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Prevalence of Bovine Mastitis in lactating Cows and Associated risk factors in and around Wolayta Soddo, Southern Ethiopia

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Abstract

A cross-sectional study was conducted to determine prevalence of dairy cow mastitis, identify predominant responsible bacteria for mastitis infection and to assess potential risk factors associated with the disease at lactating dairy cows found in Wolayta Soddo. The study was carried out from October, 2017 to March 2018 on a total of 245 milking cows by using California Mastitis test (CMT), bacteriological examination and clinical inspection of udder and teat. Of the total animals examined, 24.5 %(60 cows) had mastitis and of which 4.9% (12 cows) with clinical mastitis and 19.6 %(135 cows) with sub-clinical mastitis. Up on a bacteriological examination, 51 bacterial isolates were identified from CMT positive samples. The most common isolates were *Staphylococcus aureus* (21.56%) followed by *Streptococcus agalactia* (17.64%) and *E-coli* (13.72%) whereas, the lowest isolation rate was for *Corynebacterium bovis* (1.9%). The prevalence of mastitis in different breeds was also analyzed with an infection rate of Holstein-Fresian (8.6%), local Zebu (8.2%) and jersey (7.75%) that indicated insignificant difference (P> 0.05). Udder and teat injuries were the major predisposing factors of mastitis in current study area. Lactating cows having udder and teat injury were found to be affected at higher rate(15.5%) than those without udder and teat injury (8.9%) and it was also showed statistically strong significant difference (p-value=0.0001) in association with occurrence of mastitis. Age also affected the prevalence of mastitis and it was higher in aged (13.7%) than adult (9.7%) and young (0.8%). The association between age groups showed statistically strong significant effect (P = 0.0001) on prevalence of mastitis. The prevalence of mastitis in the current study area in relation to lactation stage was higher in early lactation (11%) than mid (6.93%) and late lactation (6.5%). In conclusion, lack of proper attention to health of the mammary glands, inadequate care in early stage of lactation, lack of breed improvement and lack of aged dairy cow replacement were important factors contributing to high prevalence of mastitis at a current study area.

Keywords: Clinical Mastitis, Sub Clinical Mastitis, Prevalence, Bacterial Isolate.

Introduction

Ethiopia currently manages the largest livestock population in Africa, estimated at 29 million cattle, 24million sheep and goats, 18 million camels, 1 million equines and 53 million poultry. These should that the country holds large potential for dairy development (Ahmed *et. al*., 2003). Dairy enterprise has shown dramatic changes in Europe and North America mainly through genetic improvements where as the development of this sector is very gradual in countries of sub-Sahara Africa like Ethiopia. In this region the low local milk production is a result of many factors including low genetic potential for milk production of indigenous breeds, the extensive and low-in put husbandry practices under which they are reared and wide spread livestock disease. The traditional small holder system which is mainly based

on indigenous bread produces 97% of the total national milk production and 75% of the commercial milk production. Accordingly, few improved exotic breed animals that mostly limited in urban and perurban area are yet not in position to satisfy the growing demand for milk production to cover the total demand has resulted improving a considerable amount of dairy products (Mohamed *et. al*., 2004).

Mostly in Ethiopia, Cows are kept to provide milk primarily for household consumption and reproduce for production of draught oxen and replacement heifers. Surplus milk is sold, usually by women, who use the regular cash income to buy household necessities or to save for festival occasions (Zewdu, 2004). Where there is access to market, dairy is preferred to met production since it makes more income to producer at most, more labor intensive and supports substantial employment in production processing and marketing (Walshet *et. al.,* 1991). The challenges represented by the expanding demand for milk and dairy products in tropical countries is great and the resultant opportunities for small holders are large (Willians *et.al.,* 1995). Furthermore, In Ethiopia 42% of total cattle for private holdings are milking cows; however, Milk production often doesn't satisfy the countries milk requirement due to multitude factors (Fekadu, 1995).

Mastitis is among various factors contributing to reduced milk production. Mastitis is a major and prevalent disease of dairy cows in Ethiopia. The total annual national milk production in the country ranges from 797,900 to 1, 197, 500 that contributed from cattle (Fekadu, 1995; FAO, 1990). However, this amount is by far below the national demand from milk and milk products in the country. Many reasons could be described for the low annual national milk yield among which mastitis is one of the most important factors. A number of reports indicated that mastitis is serious problem in the dairy industry of Ethiopia (Mekonnen *et. al.*,2005). Mastitis has been known to cause a great deal of loss or reduction of productivity, to influence the quality and quantity of milk yield, and cause culling of animals at an unacceptable age. Most estimates have shown a 30% reduction in productivity per affected quarter and 15% reduction in production cow or lactation, this making the disease one of the the most costly and serious problem affecting the dairy industry worldwide (FAO, 2003).The available information in Ethiopia indicated that bovine mastitis is one of the most frequently encountered diseases of dairy cows (Stephen *et. al*., 2001). According to (Lemma *et. al.,* 2001) of the major disease of cross breed cows in Addis Ababa milk shed, Clinical mastitis was the second most frequent disease next to reproductive disease in which 171 cows out of 556 were found to be affected. The prevalence of clinical and sub-clinical mastitis in different parts of Ethiopia range from 1.2 to 21.5% respectively. This limited studies showed that bovine mastitis is among the problems hindering dairy productivity in Ethiopia and for thus requires the development of methodologies of control program under the prevailing husbandry system. However, so far efforts have been concentrated only on the treatment of clinical cases (Hussein *et. al.,* 1997).

Generally, Milk and milk products play a very important role in feeding the rural and urban population of Ethiopia and have a high nutrition value and is daily produced, sold for cash or readily processed. It is a cash crop in the milk-shed areas that enables families to buy other foodstuffs, contributing significantly to the household food security (CSA, 2008). However, the current production of milk in the country is not satisfactory due to different constraints and mastitis is top standing problem that declines milk production at the study area. Therefore the objective of this study was to estimate the prevalence of mastitis to identify the major bacterial pathogens and associated risk factors in the current study area.

Materials and Methods

Description of Study area

The present study was conducted in southern nations and nationalities people's regional state, Wolayta Zone, at Soddo ZuriaWoreda. Wolita sodo is about 390 km away from Addis Ababa, the capital of Ethiopia and the town is located at latitude 8^0 50^0 N and longitude 37^0 45^0 E. The area covers about 63, 4471.30 haectar of land with total human population of 285,598. The altitude falls between the ranges of 7000 to 2900 meter above sea level. Damota is the highest mountain in the area which is located near Wolita soddo town with average annual rainfall of 1200millimeters and the mean annual temperature is 12^{0c}. The agro-climatic condition is *weina-dega* and the area experiences bimodal rain fall pattern with long rain season (about 75%) of the total annual rain fall extending from mid May to September and short rain season (about 25%) of the total annual rain fall extending from February to mid April. The area has three agro-ecological zones; *dega* (high land) with

altitude 1600 to 2000 to 2000 m.a.s.l and *kola* (low land) with altitude of below 1600 m.a.s.l(CSA, 2014).

Study Population

Representative Kebeles were selected to identify 245 lactating cows, 80 form Soddo dairy farm and 165 cows from urban dairy holders. The average holding capacity of cows per householder was 6 but the range is from 1 to 20. All the cows in the study were hand milked and most of them milked two times per a day during lactation period.

Study design and sampling

The study design was cross-sectional type with simple random sampling method and it was conducted from October 2017 to March 2018 in Sodozuria. The sampling included all the four quarters of the mammary glands of the cows. The methods employed in this study include physical examination of the udder and teats, California mastitis test (CMT) and bacteriological culture test.

Sample Size Determination

Sample size was determined by using expected prevalence of 20% from the previous report of thework done in the study area by Tomase(1996).Accordingly, a total sample size of 245 lactating cows from smallholder dairy herds were determined by assuming the expected prevalence of 20%, with the confidence interval of 95%, while the desired precision set was 5% for the presence of clinical mastitis, subclinical mastitis and associated risk factors based on the formula described by Thrusfield (2005).

Questionnaire survey

A questionnaire was developed and presented based on all information relating to study objectives and the data were recorded on the format prepared. Data collected include overall story of lactating cow, age, breed, stage of lactation and udder injury. The age of animals recorded asking the owner and observing the dentition by characterizing and categorizing as young (1-4 years), adults (4-6 years) and aged (>6 years). Stage of lactation was categorized as early (1^{st} to 4^{th} month), mid (4^{th} to 8^{th} month) and late (8^{th} month up to beginning of dry period).

Milk Sample collection techniques

Strict aseptic procedures were followed when collecting milk samples in order to prevent contamination with microorganisms present on the skin, udder and teats, on the hands of samplers and on the milking environment. Dirty teats were washed with clean towel and disinfected by solution and then dried. The teat and teat orifice of cows were cleaned using cotton soaked in 70% ethyl alcohol and after discarding of 3-4 streams taking 3-4milliliters following strict aseptic measure of milk for bacteriology examination by holding sterile tube nearly horizontal. The tubes were labelled with water proof marker to identify the cow and quarter from which milk was collected and transported immediately to Soddo regional veterinary medicine microbiology laboratory. The samples were stored at 4^0c and cultured within 24 hours according to (NMC, 1990; Quinn *et al.*, 1999).

Bacterial Isolation

Only those clinically mastitic milk samples and sub-clinical mastitic samples were processed for the isolation of bacteria using standard bacteriological procedures. One standard loop (0.01ml) of each milk sample was streaked on MacConkey agar and tryptone blood agar base enriched with 7% sheep blood. Plates were incubated aerobically at 37^{0C} for up to 48 hours and checked for any bacterial growth. Positive bacterial cultures were transferred into nutrient agar and further identification of the bacterial species was done on the basis of Grams reaction, colony morphology and biochemical tests and plating on selective media (Quinine et al., 2002).

Data management and statistical analysis

The collected data in Microsoft excel spread sheet were transferred to SPSS version 20.0for analysis.. The prevalence of mastitis (clinical and sub-clinical mastitis) calculated by using percentage values and possible association of disease with risk factors was analysed by using Chi-square test and predictive value (P-value). Significance of risk factors on the evidence of mastitis in lactating cows was tested to check presence of significant association between CMT positive and risk factors by considering $p<0.05$ as statistically significant.

Results

Prevalence of Mastitis: Out of the total 245 cows examined, 60(24.4%) of the cows had abnormalities in udder and milk as evidence of mastitis. Furthermore, 12 (4.9%) of them had clinical mastitis and 48(19.6%) had sub-clinical mastitis. In the clinically infected cows, there were visible abnormalities in the milk or the udder. Secretion from affected quarter varied considerably from purulent, blood tinged and malodorous to watery form. There were also udder swelling, hardness of the affected quarter, pain, fever, depression and in-appetence on clinically infected cows at the study area (Table 1).

Tab 1: The prevalence of clinical and sub clinical mastitis

Types of mastitis	Infected	Prevalence (%)
Clinical	12	4.90
Sub-clinical	48	19.60
Total	60	24.50

Potential Risk Factors:

Clinical examination and screening test results indicated an overall prevalence of 8.6% (21/245) in Holstein Friesian, 7.75% (19/245) Jersey and8.2% (20/245) Local Zebu. As the results shows, Holstein Friesian was most affected than local zebu and jersey cows at the study area. The prevalence in the current three breeds was not statistically significant (P> 0.05) (Table 2). The prevalence of mastitis on early lactating cows were higher than the mid and late lactation stage with the ratio of 11%, 6.93% and 6.5% respectively. The relationship between lactation stages and prevalence of mastitis was statically insignificant (p>0.05) as described in (Table 3). The majority of mastitis case occurred in older cows (13.7%) than young (0.8%) and Adult (9.7%) according to their age. The study reveals that the cows with age of six years and above were most commonly affected by Mastitis following the cows between four and six years old. There was strong statistical significant difference (p<0.05) in the age group with respect to occurrence of mastitis (Table 4). Animals having udder and teat injury were found to be affected at higher rate (15%) than those without udder and teat injury (8.9%) and the difference is statistically significant (Table 5).

Table 2: The prevalence of mastitis among breed

Mastitis status in breeds							
	N	Holstein Friesian		Jersey		Local Zebu	
		n	%	n	%	n	%
Positive	60	21	8.60	19	7.75	20	8.20
Negative	185	29	11.83	41	16.73	115	46.9
Total	245	50		60		135	
x^2 = 18.14 ; P- value = 0.0673							

Table 3: The prevalence of bovine mastitis compared by lactation stage.

Stage of lactation	n	Infected	Percentage	X^2	P- value
Early	110	27	11		
Mid	51	17	6.93	314.938	0.064
Late	84	16	6.5		
Total	245	60	24		

Table 4: Prevalence of mastitis influenced by age group

Age	n	Infected	Percentage	X2	P- value
Young 1-4 years	38	2	0.8		
Adult >4 and <6	112	24	9.7	17.117	0.0001
Aged 6 years	95	34	13.7		
Total	245	60	24		

Table 5: The Prevalence of mastitis associated with udder and teat injury

Injury	n	Infected	Prevalence	X^2	P-value
Present	70	38	15.5%		
Absent	175	22	8.9%	51.593	0.0001
Total	245	60	24%		

Bacteriological Analysis

A total of 980 quarters were investigated from 245 milking cows in the study area. All milk samples which were taken from mastitis positive cows either clinically or sub-clinically were subjected to bacteriological analysis. Accordingly 173 milk samples from positive quarter were cultured and growth of bacteria on culture media observed only in 51 (29.5%) milk samples. However, sample taken from 122 (70.5%) positive quarters of cows cultured in the media was not showed any growth of bacteria (Table 6). As the summary of (Table 7) indicates, total number of isolates revealed as clinical and sub-clinical mastitic cases were 20 and 31 respectively. Of the 20 isolates from clinical cases, *staphylococcus aureus* and *Streptococcus agalactia* had similar ratio each 5(9.8%), *Streptococcus dysgalactiae* and *E-coli* each had 2 (3.9%) and *Klebsiella pneumoniae* 1(1.9%). Micrococcus and Corynebacterium were not observed in clinical cases. The general relative isolation rates of each bacteria in mastitic cases were 21%, 17.64%, 13.72%, 9.8%, 9.8%, 7.8%, 3.9%, 3.9%, 3.9% and 1.9% for *Staphylococcus aureus, Streptococcus agalactia, E-coli, Klebsiella pneumoniae, Staphylococcus intermedius, Streptococcus dysgalactiae, Staphylococcus epidermadus, Staphylococcus hyicus, Micrococcus* and *Corynebacterium bovis* respectively. According to the finding, *Staphylococcus aureus, Streptococcus agalactia* and *E-coli* were common isolates from clinical and sub clinical cases of the mastitis. *Staphylococcus aureus* was the predominant isolate whereas *corynebacterium bovis* contribute the least frequency (Table 7).

Table 6:Occurrence of mastitis in quarter level

CMT positive quarters	Growthon culture media		Nogrowthon culture media	
	Number	%	Number	%
173	51	29.5	122	70.5

Table 7: Relative frequency of bacterial species from clinical and subclinical mastitis cases by bacteriological examination

Types of bacterial species	Number of isolates		Total (%)
	Clinical mastitis (%)	Sub-clinical mastitis (%)	
Staphylococcus aureus	5(9.8)	6(11)	11(21.57)
staphylococcus intermedius	-	5(9.8)	5(9.8)
staphylococcus epidermadus	-	2(3.9)	2(3.9)
Staphylococcus hyicus	-	2(3.9)	2(3.9)
Streptococcus agalactia	5(9.8)	4(7.8)	9(17.64)
Streptococcus dysgalactiae	2(3.9)	2(3.9)	4(7.8)
E. coli	2(3.9)	5(9.8)	7(13.72)
Klebsiella pneumonia	1(1.9)	4(7.8)	5(9.8)
Micrococcus	-	2(1.9)	2(3.9)
Corynebacterium bovis	-	1(1.9)	1(1.9)
Total	20	31	51(100)

In the current study area, 120 farmers in smallholder private farm and Soddo state dairy farm were interviewed for Mastitis case. All the owners of the farm practiced hand milking on their smallhold farm and some familiar farmers with mastatic case occurance detected infected cows only by observing milk change and udder inflamationon their cows. Most of the respondents were not familiar with the disease at all and only ten respondents (8.3%) of them had the knowledge about the appearance of the clinical form of mastitis and six respondents (5%) had cow with one or more blind teat but they had no knowledge about the cause.

Discussion

A total of 245 cows, 135 lactating local zebu, 50 Holstein and 60 jerseys from private smallholder farmers and Soddo dairy farm were investigated cross-sectionally and revealed overall prevalence of 24.5%.This finding was found lower than the previous finding in and around Addis Ababa by (Hundura *et. al.*, 2005) who reported the prevalence of 52.78% and slightly similar with the report from Wolayta Sddo which was 27.3% by (Tolosa *et. al.*, 2009). However, the current finding was higher than the prevalence of 7.2%, 8.7% and 11.1%reported by (Nesru *et. al*., 1997) in central Ethiopia and (Bishi, 1998) in and around Addis Ababaand (Wudu,1999) around Mekelle respectively.

The present finding showed clinical mastitic cases with the prevalence level of 4.9% in Holstein, local Zebu breeds and gersey. The clinical mastaitis prevalence in this study was slightly comparable with that of (Bishi,1998) who reported the prevalence of 5.3%, in Addis Ababa Ethiopia and it was lower than the report of(Tolosa *et. al*., 2009) who recorded the prevalence of 9.5% at Wolayta Soddo. The prevalence of 25.1% and 16.11% were also reported by (Workineh *e.t al*., 2002) and (Hundra *et. al*., 2005) respectively in and around Addis Ababa, Ethiopia, that was far higher than the current finding. The difference in results could be due to difference in management system between the farms and the complexity of the disease.

Sub-clinical mastitis was higher compared to clinical mastitis in all three breeds with the rato of 19.6% and 4.9% respectively. The prevalence of sub-clinical mastitis at cow level based on CMT in the present study (19.6%) was higher than the finding of (Nesru*et. al*., 1997) who reported 1.9% in central Ethiopia; and (Bishi, 1998) who reported the prevalence of 3.2% in and around Addis Ababa and (Tolosa *et. al*., 2009) who reported the prevalence of 17.5% in wolayta Soddo. In Ethiopia, the sub-clinical form of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases (Hussein *et. al*., 1997) whereas the high economic loss could come from sub-clinical mastitis.

In this study as well as in other similar studies, overwhelming cases of mastitis were sub-clinical compared to clinical mastitis in all breeds (Kassa *et al*., 1999; Hussein, 1999; Workineh *et al*., 2002; Kerro and Tareke, 2003). Due tocomplexty of disease involving interactions of several factors, mainly of management environment, and factors relating to animal and causative organisms, the prevalence of mastaitis is expected to vary from place to place. The significant difference in prevalence of mastitis between husbandry practices could be attributed to the variation in hygienic standards of dairy environment and milking conditions, as well as genetic variation in disease resistance among the breeds (Radostits *et al*. 1994).

Among the risk factors considered to had effect on the occurrence of mastitis in age group (young 0.8%, Adult 9.7% and Aged 13.7%), and under injury (present 15.5% and Absent 8.9%) had been statistical significance ($P<0.05$) thus agree with the finding of (Bedane *et. al*., 2012) at south Ethiopia. There was higher prevalence of mastaitis on early lactation stage (11%) than mid (6.93) and late (6.5) lactation stage in the current study area with no statistical significance that disagree with the report of (Bedane *et. al*., 2012) who reported statistical siginificance between lactation stage and mastaitic cases. However, the current finding agrees with that of (Prem *et al*., 1995) who reported high incidence 12% in cow during early stage of lactation in India.. Absence of dry cow therapy regime could possibly be among the major factors contributing to high prevalence at early lactation. During a dry period due to the low bactericidal and bacteriostatic qualities of milk, the pathogens can easily penetrate in to the teat canal and multiply. This can be carried over in to the post-parturient period and ultimately develop in to clinical mastitis.

Breed influence on prevalence of mastitis could be attributed to the difference in certain physiological and anatomical characteristics of the mammary glands. Occurrence of mastitis may be influenced by some heritable characteristics such as capacity of milk production, teat structures, and udder conformation (Abaineh, 1997). The current findings indicates that Holestianfresian was mostly affected bereed with ratio of (8.6%) than that of Local zebu (8.2%) and Jersey (7.75) breeds. Thes finding was lower than the finding of (Wubeshet*et. al*., 2017) who reported 28.6% prevalence on Holestianfresian at Wolita sodo town. Inadequate hygienic condition of dairy environment, poor animal health service, and lack of proper attention to health of the mammary gland were important predisposing factors of mastitis in the area. Adequate housing with proper sanitation and regular screening for early detection and treatment, follow up of chronic cases, culling of older cows with repeated attacks, and prompt treatment of teat or udder injuries are recommended to alleviate the problem (Shimelis, 1990).

Out of 980 quarters, 173(17.65%) were found to be affected with clinical and sub-clinical mastitis. In this study, *staphylococcus aurous* was the predominant pathogen involved (21.57%) in both clinical and sub-clinical mastitis. The high level isolation of *Staphylooccus aureus* (21.57%) in this study closely agrees with the findings of (Bishi, 1998) in Ethiopia who reported 24% around Addis Ababa. In contrast to the current finding, in Poland, a survey of mastitis in dairy herds of small-type farms in the Lublin region, *Staphylococcus aureus* was isolated at a higher rate (36.6%) compared to other pathogens (Krukowski *et. al*., 2000). The isolation rate of *streptococcus agalactia* (17.64%) in this study was the second prevalent isolate next to *staphylococcus aureus* and the finding was higher than the report of (Bishi, 1998) who reported 9% prevalence in Addis Ababa. However, the present finding was lower than that of (Workineh *et al*., 2002); (Kerro and Tareke2003) where *Streptococcus agalactia* accounted for 39.2% and 40.5% of the isolates respectively in Addis Abba and Southern Ethiopia. The relatively high prevalence of *Streptococcus agalactia* in this study could be associated with absence of dry cow therapy. Other dominant pathogens isolated from clinical and sub-clinical mastitis in this study includes *E.coli, Klebsiela pneumoniae, Staphlococcus intermidus* and *Streptococcus dyisagalctia*, 13.72, 9.8%, 9.8%, 7.8% respectively. Other less frequently encountered pathogens includes *Staphylocous epidermadus, Staphylococus hyicus* and *Micrococcus* with a rate of3.9% for each and *Cornybacterium bovis* with a rate of 1.96%.

Conclusion and Recommendations

The current study attempted to quantify mastitis in Wolayta Soddo smallhold farms, isolate and determined the prevalence of the disease and major pathogens involved and investigated some of risk factors associated with infection. Sub-clinical mastitis was more important when compared to clinical mastitis. There was several factors affecting the

prevalence of the mastitis in Wolayta Soddo private small holder dairy farm and Soddo state dairy farm. Some of the selected risk factors included wasbreed, age, stage of lactation and udder injury. The pathogens found involved were *Staphylococcus aureus, Streptococcus agalactia, Streptococcus Dysgalactiae, Staphylococcus epidermadus, Staphylococcus hyicus,Micococcus species, Corynebacterium bovis, E. coli* and *StaphylococcusIntermidius.* Among these, the most frequent isolates were *Staphylococcus aureus, Streptococcus agalactia, E-coli, Staphylococcus intermidius* and *Klebsiella pneumonia*. Least frequently encountered species were *Corrynbacterium bovies*. In this study, coliform bacteria or environmental pathogens and contagious pathogens were isolated from clinical and sub-clinical form of mastitis. Therefore in order to prevent such kind of devastating disease in dairy farm, the following recommendations are forwarded.

- Prompt treatment of the injuries and wounds on udder and teats to be done and their prevention to avoid the occurrence of infection of mastitis.
- Regular monitoring of sub-clinical mastitis and its treatment during drying off period to prevent clinical mastitis after calving and cure summer mastitis.
- Education and demonstration of good dairy management, milking hygiene and correct method of hand milking to the dairy famers through mass media like radio and TV programs. and careful milking practice such as use of single towel for each cow, disinfecting hands before milking and between milking and milking infected cows last principle should be followed.
- Further investigation, on dairy cows subclinical mastitis as well as mastitis causing pathogen at molecular level and their association with potential risk factors and improving the breeds of the dairy cows at the study area are recommended.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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