

# Epidemiology of Bovine Mastitis and Associated Risk Factors in East Shewa Zone of Oromia Regional State, Ethiopia

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**Review Article**

Volume 2 Issue 2

**Received Date:** February 22, 2017

**Published Date:** March 21, 2017

## Abstract

A cross sectional study was conducted in selected districts of East Shewa Zone of Oromia Regional State from November 2013 to April 2014 on dairy cows to determine the prevalence of mastitis and determinant of its major risk factors. The study was done on 250 lactating cows, of which 109 were local and 141 cross breed by using clinical examination, California mastitis test (CMT) and culture. Of these 108 (43.2 %) were positive by clinical examination and CMT for clinical and sub clinical mastitis, with prevalence of 4 % and 38.8 %, respectively. All the potential risk factors considered in this study namely, parity ( $p < 0.05$ ), breed ( $p < 0.05$ ), age ( $p < 0.05$ ), stage of lactation ( $p < 0.05$ ), breed ( $p < 0.05$ ) and husbandry ( $p < 0.05$ ) showed a significant effect on prevalence of mastitis in present study. Thus, high prevalence of mastitis was observed in older cows  $>7$  years and cows with parity  $>7$  but high prevalence was obtained on early and late stage of lactation. The study also revealed that cross breed lactating cows and those managed intensively were susceptible to mastitis. The highly prevalent bacteria isolated were *Staphylococcus aureus* (46 %) followed by *Streptococcus agalactiae* which accounts (19.4 %), and others were *Staphylococcus intermedium*, *E.coli* and *Corynebacterial* species with respective proportion of 16.6, 10.9 and 7.1%. The present study also revealed that mastitis is the major problem in smallholder dairy farms in the study area specially the sub clinical form, so that creation of awareness about the importance and prevention of subclinical mastitis among smallholder dairy farmers, milking infected animals and periodic monitoring of infection status of the udder is recommended.

**Keywords:** Prevalence; Subclinical Mastitis; Risk; East Shewa

## Introduction

In Ethiopia, livestock represents a major national resource and form an integral part of agricultural production system [1] of which dairy sector has large potential market for development and its growth is expected to continue for the next one to two decades due to the growth of income, increased urbanization

and policy improvement. Thus, the development of smallholder dairy production sector in the country contributes in poverty alleviation by generating income through self-employment. Cows represent the largest proportion of the cattle population of the country; however, the milk production does not satisfy the country's requirements due to multitude factors. Disease of the mammary gland known as mastitis is the

major contributing factor in the reduction of milk production [2], and it is among the most important diseases in dairy animals with worldwide distribution [3].

Mastitis has a negative effect on the image of dairy production chain. Although these factors have an economic impact on dairy production, more losses may occur on the dairy producer level, due to culling of the infected cow, veterinary costs, extra-labor costs, disposed milk, reduced slaughter value, and reduced reproductive performance [4,5]. Mastitis is a multi factorial disease. As such, its incidence depends on exposure to pathogens, effectiveness of udder defense mechanisms, and presence of environmental risk factors, as well as interactions between these factors [6].

Most commonly, mastitis begins as a result of penetration of the teat duct by pathogenic bacteria [7]. However, some viral diseases affecting the epithelium of the teat orifice are mentioned to result in or predispose to mastitis [8]. The most commonly incriminated and reported causes of mastitis include *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus* species other than *Streptococcus agalactiae*, *coagulase negative Staphylococci species*, *Escherichia coli*, *Micrococcus species*, *Corynebacterium species*, *Bacillus species*, *Pasteurella species*, *Klebsiella species*, *Mycoplasma species* and *Nocardia species* [9-11].

Mastitis can be either clinical (CM) that give rise to visible symptoms or subclinical. Mild CM causes flakes or clots in the milk, whereas severe cases are associated with heat, swelling and discoloration of the udder, as well as abnormal secretion. Severe CM can also exhibit systemic reactions, such as fever and loss of appetite. Mastitis can exist in the absence of visible signs of infection, and is then referred to as subclinical mastitis (SCM). SCM is the most prevalent form of mastitis [12]. The greatest risk of acquiring mastitis occurs in the first 50 days of lactation and in the early part of the dry period. The risk of clinical mastitis also increases with increasing parity [10]. In practice, whether a case of mastitis is classified as clinical or subclinical often depends on how carefully the cow is observed when diagnosis is made [13]. Transmission occurs mainly at milking time through contaminated milking machines, clothes and hands of milkers or machine operators. Contagious mastitis is transmitted from cow to cow by pathogens for which the udder is the primary reservoir. It tends to be sub-clinical in nature. Bacteria that live on the skin of the teat and inside the udder [14] mostly cause it. The primary reservoir of contagious pathogens is the mammary gland itself [15]. This disease also poses the risk for the transmission of zoonotic diseases like tuberculosis, brucellosis, leptospirosis and streptococcal sore throat to human beings [5]. Treating subclinical mastitis with antimicrobials during lactation

is seldom economical, because of high treatment costs and generally poor efficacy. All mastitis treatment should be evidence-based, i.e., the efficacy of each product and treatment length should be demonstrated by scientific studies. Because of the diverse bacterial etiologies of the disease, a variety of control method involving hygiene prior to, during and after milking are used to minimize exposure of cows to mastitis causing organisms. Despite these procedures, new cases of mastitis invariably occur and antimicrobial therapy plays a role in the control of bovine mastitis [16]. This study aimed to assess contribution of risks factors to mastitis prevalence and to evaluate the link between selected risk factors and the prevalence of bovine mastitis in East shewa zone of oromia region.

## Materials and Methods

### Study Area and period

The study was conducted from November 2013 to April 2014, in Oromia Region; East shewa Zone. The area is located at 74km from Addis Ababa and 27 km from Bishoftu, at Longitude between 38°56'E-39 °17'E and Latitude 8°34'N - 8°34'N. The average Elevation of the area is 1780m above sea level. The area have average annual rain fall 969.35mm, and mean annual temperature of 20.4 °C. Management system of the animals is extensive production system. The main farming system is mixed farming and sheep are the predominant animal species kept in the area. Traditional housing and grazing of natural pasture are the predominant husbandry practice. The total animal population of the woreda is 752,612; among which 99,862 are cattles, 26,693 sheep, 25,136 goats, and 64,094 poultry.

**Study Design and Population:** A cross-sectional study was conducted on randomly selected local and cross breed lactating cows, selected from different peasant associations (PAs). Simple random sampling was considered to select the animals.

**Sample Size Determination:** The sample size was determined at 95% confidence level, 5% precision. Thus, the sample size value was read from Thrusfield [17] sample size Table to be 380 animals. But due to lack of cooperation in different peasant associations which is more of extensive system, it is impossible to get lactating cows at a time. As a result 250 lactating cows were included in the investigation.

$$n = 1.962 P_{exp} (1 - P_{exp}) / d^2$$

Where; n= required sample size

$P_{exp}$  = expected prevalence

d= required precision

### Sample Collection

**Physical examination of the udder:** We first examine the udder through visually and then palpation to detect abnormalities (fibrosis, cardinal signs of inflammation); for cows with clinical mastitis we also proceed on other examination (Rectal temperature and Auscultation) to check systemic involvement.

**Preparation of udder and teats:** The udder, especially teats was cleaned and washed with tap water, dried with a dry towel and dipped using dipping solution before and after sample milk collection to avoid sample contamination and afterward entry of germs in the udder.

**Sample collection, handling and storages:** Milk samples were collected by a standard milk sampling techniques. To reduce contamination of teats ends during sample collection, the near teats first followed by the far once. After discarding 3 first stream of milk, sample milk was collected into sterile test tube placed in racks for ease handling and transported in an ice (cooler box) to the laboratory and stored at 4°C for a maximum of 24 hour until inoculated on a standard bacteriological media [18].

**California Mastitis Teat (CMT):** CMT results was used to diagnose subclinical mastitis considering the nature of coagulation and viscosity of the mixture (milk and CMT reagent), which show the presence and severity of the infection, respectively [10]. The positive samples show gel formation within a few seconds. The result was scored based on the gel formation and categorized as negative if there was no gel formation, or positive if there was gel formation. If at least one quarter was positive then the cow was taken as mastitic.

**Bacteriological examination of milk samples:** Milk samples were bacteriologically examined according to the procedures employed by Quinn et al. [19]. A loop full of milk sample collected from each infected quarter was inoculated separately on to MacConkey agar and blood agar base. The inoculated plates were then incubated aerobically at 37°C for 24 to 48 hours. Identification of the bacteria on primary culture was on the basis of colony morphology, haemolytic characteristics, Gram stain reaction including shape and arrangements of the bacteria. The growth was then transferred to nutrient agar for further biochemical tests.

**Data analysis:** The data collected from the study area including different risk factors (age, parity number, stage of lactation, cleanness of teat, breed and husbandry system) and other examination were recorded in the format developed for this purpose and later entered and analyzed using SPSS 16 version.

### Results

**Prevalence of Mastitis:** The prevalence of mastitis at cow 43% (108/250). From the total of 250 lactating cows examined 12 (4%) had clinical and 96 (38.8%) had subclinical mastitis, (Table 1).

Type of Mastitis	Number of Positive	Prevalence of Mastitis Positive %
Clinical mastitis	12	4
Subclinical mastitis	96	38.8
<b>Total</b>	<b>108</b>	<b>43</b>

Table 1: Prevalence of clinical and sub clinical mastitis at cow (n=250)

### Associated Risk Factors

Table 2 shows 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 ( $p < 0.05$ ), husbandry system ( $p < 0.05$ ), parity number ( $p < 0.05$ ) and lactation stages ( $p < 0.05$ ). Cows in their early and late stage of lactation suffered most from mastitis compared to cows in their mid stage of lactation. Cows managed intensively ( $p < 0.05$ ) was highly susceptible to mastitis when compared to those managed extensively with respective number and percent of 85(73.9%) and 23 (17.03%) among the totally examined animals. Mastitis prevalence also increased with an increase in cow dirtiness

Cow dirtiness	Number	Mastitis		%Mastitis positive
		Negative	Positive	
Very dirty	47	19	28	11.2
Moderately dirty	122	77	45	18
Slightly dirty	81	46	35	14
<b>Total</b>	<b>250</b>	<b>142</b>	<b>108</b>	<b>43.2</b>

Table 2: Mastitis Prevalence In Relation To Cow Cleanness Scores.

Risk factors	Group	Number	Positive	X <sup>2</sup>	p-value
Age(yr)	4-Feb	35	21	8.75	0
	6-Apr	154	36		
	>7	61	51		
Lactation stage (month)	3-Jan	63	49	8.01	0
	6-Apr	82	38		
	>7	105	21		
Parity	3-Jan	45	18	5.3	0.016
	6-Apr	102	25		
	>7	103	65		
Breed	cross	109	73	15.55	0
	local	141	35		
Husbandry	Intensive	115	65		0.0001
	Extensive	135	43		

Table 3: Prevalence of mastitis in relation to different risk factors.

### Bacteriological Results

Up on culturing milk from 280 positive quarters on bacteriological media 211 different isolates were obtained. Among the total isolated *Staphylococcus aureus* accounted for the highest percent (46 %) followed by *Streptococcus agalactiae* which accounted for 19.4 %. Others were *E. coli* which was (10.9%), *Staphylococcus intermedium* (16.6%) and *Corynebacterial* species which was 7.1% (Table 4).

Bacterial species	Number of isolates (n=280)	% Prevalence
<i>Streptococcus agalactiae</i>	41	19.4
<i>Staphylococcus aureus</i>	97	46
<i>Escherichia coli</i>	23	10.9
<i>Staphylococcus intermedium</i>	35	16.6
<i>Corynebacterial species</i>	15	7.1
Total	211	75.4

Table 4: Bacterial Species Isolated From Bovine Mastitis

### Discussion

The overall prevalence of mastitis 43.2% at cow level was comparable to the findings of Workineh et al. [11], Bishi [20], Mungube et al. [21] and Fekadu [22] who reported 40.4% in southern Ethiopia, 38.2% in Adami-Tulu central Ethiopia, 39.8% in and around Addis Ababa. However, it is relatively lower than the study of Lakew et al. [23] and Zeryehun et al. [24] that got 71.1% from Holeta, 52.8% from Sebeta, 64.6% from Assela and

74.7% around Addis Ababa respectively. The prevalence of sub clinical mastitis reported during the study was 38.8% at cow level which was in close agreement with prevalence reported by Abaineh and Sintayehu [25], Sori et al. [15] and Lakew et al. [23], with respective percent of 37.2, 34.6, 36.7 and 38.1% while the prevalence of clinical mastitis at cow level (5.3%) was in agreement with the finding of Enyew [16] with recorded prevalence of (3.9%) from Bahir Dar, Ethiopia. In general, subclinical mastitis has been reported to be higher than clinical mastitis owing to the defence mechanism of the udder, which reduces the severity of the disease [9]. Risk of mastitis increased with age and parity. This observation is in agreement with the reports of Biffa et al. [26], Mungube et al. [21] and Kerro and Tareke [27]. Mastitis prevalence was high in early and late stage of lactation. This result is comparable with observations of Biffa et al. [26] and Kerro and Tareke [27] who reported high prevalence of mastitis in the early and late stage of lactation. Breed and husbandry system also showed significant influence on the prevalence of mastitis in which high prevalence of mastitis was observed in cross breed and those managed intensively compared to local cows and with those managed extensively which is in agreement with other findings of previous study. Microbiological examination of milk from lactating dairy cows showed the highly prevalent bacterium in the study area was *Staphylococcus aureus* which accounts 46 %. This result is in line with the findings of Workineh et al. [11], Bedada and Hiko [28], Deogo and Tareke [27] who reported 39.2, 39.1 and 40.3% *Staphylococcus aureus* isolates at Addis Ababa, Assela and Southern Ethiopia, respectively. However it was higher than some previous report of Bitaw et al. [29,30] who found 20.3% in dairy farms in Bahir Dar town, Bishi [20] who reported 9% prevalence in Addis Ababa. The possible difference for this might be due to *Staphylococcus aureus* is a

contagious pathogen transmitted from one cow to another or individual by contact with animals during unhygienic milking procedures [5]. The next most prevalent bacteria isolated was *Streptococcus agalactiae* (19.4%) followed by *Staphylococcus intermedium*, *E. coli* and the least of bacterial isolated in the area was coryne bacterial species with their respective percent of 16.6, 10.9 and 7.1.

### Conclusion and Recommendation

The overall prevalence of mastitis in East shewa zone was 43.2%. This indicates that mastitis is a serious problem across herds in this area. The main risk factors associated with the prevalence of mastitis, in order of importance, parity, age, and cow dirtiness, stage of lactation, breed and husbandry. Mastitis infections were largely caused by *Staph. aureus* (46%) and environmental staphylococci (16.6%). These micro-organisms are associated with poor hygiene and contamination of udders and milking equipment with soil and faeces.

It is recommended that dairy farmers in East shewa zone introduce mastitis control programmers, particularly teat dipping, dry cow therapy and effective treatment of clinical mastitis, to reduce the high prevalence of mastitis in their herds. Moreover, extension services and training programs aiming at creation of awareness about the importance and prevention of subclinical mastitis among smallholder dairy farmers, milking infected animals and their respective quarters at last and periodic monitoring of infection status of the udder is recommended.

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