

# Prevalence of Bovine Mastitis and Assessment of Risk Factors in and Around Wolayta Sodo, Ethiopia

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**Abstract:** The purpose of this cross-sectional study was to estimate the prevalence of mastitis and to assess the associated risk factors in dairy breeds (jersey, indigenous zebu, and high grade Holstein) in the Woliata Soddo area and surrounding areas from November 2013 to May 2014 at the Soddo regional veterinary laboratory. A clinical examination and Californian Mastitis Test (CMT) were conducted on 386 nursing cows, including 216 indigenous zebu, 88 jersey, and 82 high grade Holsteins. The patients who tested positive for both clinical and subclinical mastitis were then tested for bacteria. Among the 134 animals tested, 34.7% tested positive for clinical mastitis and 61.19 percent tested positive for subclinical mastitis. A total of 21.94 percent of the 966 quarters tested positive for mastitis. Mastitis was much more common in high grade Holstein cows (54.8%), jersey cows (44.3%), and indigenous zebus (23.1%) compared to other breeds ( $P < 0.000$ ). Regarding quarters, however, no discernible variation existed between breeds. Mastitis was most common between 1 and 120 days into early lactation (43.8%), between 120 and 240 days into mid-lactation (32.8%), and beyond 240 days into end-lactation (23.8%). Additionally, the difference between lactation was statistically significant ( $P < 0.000$ ). There was a positive correlation between parity and mastitis prevalence ( $r = 0.8$ ), with the greatest incidence occurring at lactation stage 6 and thereafter. Microbiologic analysis was performed on 134 udder quarter samples; 111 (82.8%) were found to be culturally positive, whereas 23 (17.2%) were found to be negative. Out of the 124 bacteria that were recovered in positive samples, 43.54% were Staphylococcus, 28.89% were Streptococcus, and 19.35% were Coliform. Findings informed calls for further education of dairy cow owners and calls for additional research into financial losses.

**Keywords:** Woliata Soddo, Bovine Mastitis, Prevalence, CMT, Bacteriological Examination, Risk Factors

## 1. Introduction

Injurious substances such as pathogenic microbes, trauma, and chemical irritants may cause mammary gland inflammation, which is known as mastitis. Mastitis in dairy cows is always caused by microorganisms, most often bacteria but sometimes yeast, mycoplasma, and, on rare occasions, algae. Staphylococci, Streptococci, and a few other gram-negative bacteria are the most common infecting microorganisms. The teat end may get infected with infectious bacteria that are found in the environment, such as coliforms and some streptococci species [1].

Although moderate or severe clinical manifestations are possible, mastitis is often subclinical in character. Host, agent, and environmental factors all play intricate roles in illness etiology and progression [1, 2]. Economically significant decreases in output and productivity are caused by mastitis. Mastitis milk may cause significant financial losses because of

the risks associated with treating the infection, throwing out antibiotic-treated milk, decreasing the market value of milk, and even mortality from inflammations [1, 3].

Food poisoning, disruption of production processes, and the transmission of zoonotic diseases including TB, brucellosis, and streptococcal sore throat are all possible outcomes of milk produced by mastitic animals. Also, the public health issue of antibiotic residue infections and mastitis-treated

animals' milk is a problem [1]. In Ethiopia, the limited information available [8] indicates that bacteria mastitis is one of the major facing all dairy farms in the Country. Moreover, Mohammed [9] in his study on the bacterial causes of bovine mastitis in Zebu, Holstein dairy cows of Wondogenet all of southern Ethiopia found a quarter infection rate of 16% by potentially pathogenic bacteria however, economic losses due to mastitis in Ethiopia are not

known. According to [10] in their assessment of 10 state dairy farms more than 10% of farms in most farms in Ethiopia have at least one blind quarter. In addition, [11] in their assessment of 10 state dairy farms near Addis Ababa point out that mastitis accounts for major economic losses. In general, regarding the occurrence and distribution of major causative agents of the disease, in Ethiopia in general, and in southern regions in particular, is inadequate, moreover, not enough investigations have been carried out to ascertain the effects of different risk factors on the prevalence of mastitis. Therefore, by considering all the above information this study set out the following objectives: to estimate the prevalence of bovine mastitis in Woliata Soddo; to isolate and identify major potentially pathogenic bacteria that cause bovine mastitis and to recommend the appropriate control and prevention methods that are applicable in the study areas.

## **2. Materials and Methods**

### **2.1. Study Area**

2.2. From November 2013 to May 2014, researchers at the regional veterinary laboratory in Woliata Soddo town conducted the study. Situated in the SNNPR, about 383 kilometers from Addis Ababa, lies the town of Woliata Soddo. Damot gale woreda forms the northern boundary, Humbo Woreda the southern one, Damotwoide the eastern one, and Damotsore the western one. It gets 100–1200 mm of rain each year and 25–35°C in temperature, and its height spans from 1650–2980 m.a.s.l. The Woliata Soddo zone is located in a woinadega (mid altitude) region with an elevation below 1600 meters. The animal population in this area includes around 128,919 cattle, 29,191 sheep, 4,606 horses, and 55,272 chickens [12]. Study Population and Their Management System

Here, we studied indigenous zebus from around Sodo, jersey cows from Sodo dairy farm, and high grades Holstein Frisian from the dairy farm in the sodo area and in the Sodo town. Indigenous zebu animals in peasant farm are reared mainly for meat, milk production, and the generation of cash income and the provision of draft power. They are not supplemented except during dry season when farmers offer their animals straw of various crops and pasture grasses in acut, and carry form. They usually

graze on communal pastures where transmission of contagious disease is facilitated. The dairy farm at Soddo had relatively improved management system; jersey cows were housed in a free stall in which each animal was tied by chain. Pregnant cows were transferred to a maternity house a few days before parturition, generally without any preparation. Calves aging up to 6 months were penned separately. Cows were allowed to graze on native or improved pastures or both growth on the fenced grazing land of the farm and watered with hay or concentrates mainly during the dry season. Generally, problem associated with inadequate nutrition were uncommon, milking was conducted twice a day

### **2.3. Study Methodology**

#### **2.3.1. Study Design**

A cross sectional study design was conducted from November 2011 to May 2012.

#### **2.3.2. Sample Size Determination and Sampling Technique**

A total of 386 cows, of which 216 indigenous zebu, 88 jerseys and 82 high grade Holstein Friesian were sampled using the formula on [13]. The cattle were grouped into lactating cow (in milk), breed as indigenous zebu, jersey and high grade Holstein Friesian, lactation stage into early lactation (1-120 days) mid lactation (121-240 days) late lactation (above 240 days) and parity number as 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> and above 6. Milking sample was collected hygienically after each quarter was washed with clean water and soap and then cleaned with a swab dipped in 70% alcohol while extruding the external sphincter by pressure to ensure that dirt and wax were removed from the orifice. Ten ml of milk were collected in a test tube and held in oblique manner to prevent contamination of samples by falling particles.

### **2.4. Data Collection**

During the sampling of animals for the present study, the breed, parity number, status of mammary gland, stage of lactation or lactation period were noted, and so were clinical findings (e.g., palpable and visible abnormalities of the udder, nature and appearance of milk secretion).

*Questionnaire:* A preliminary survey was conducted in the specified study areas and adjoining localities during initial period of the

study. The questionnaire targeted mainly dairy farms and livestock owners; it was aimed at generating basic information on livestock management system, nutrition, hygienic practices, disease detection, housing, prevention and control measures.

**Diagnosis of mastitis:** Although mastitis in an individual cow may be obvious, it is more often a sub clinical case or disease and clinical. Therefore tests for the detection of changes in the udder or the milk caused by mastitis are necessary. In this study criteria for the detection of mastitis included: 1) Physical examination of udder quarter for signs of inflammation by a blood agar plates containing 7% of sheep blood, Maconkey agar plates or Edward's agar plates. Inoculation of plates performed with a 0.01 ml wire loop. The original milk samples were also incubated at the same temperature and cultured similarly 24 hours after bacterial growth was identified and recorded, at 24 and 48 hours of incubation.

At the end of this period, identification of isolated bacterial was made on the basis of morphology, color and size of the colonies, presence or absence of hemolysis and gram stain. The identification of bacterial isolates up to the species level was made by sub culturing a single pure colony and conducting the following tests:

*Staphylococcus* species and *micrococci* were identified and differentiated by gram staining, colony morphology, catalase test, tube coagulase test using human plasma, ability to produce hemolysis, ability to ferment maltose, and manitol.

*Streptococcus* species were identified and differentiated by colony morphology catalase test, growth characteristic on Edward's media, ability to produce hemolysis CAMP test.

Coliform species were identified and differentiated by colony morphology on the Maconkey agar; gram staining, motility test, indole test, triple sugar iron agar for the detection of lactose and glucose fermentation and hydrogen gas production.

### 2.5. Statistical Analysis

The data collected was filled into microsoft excel and then analyzed with Chi-square and P-value using SPSS version 20.

## 3. Result

thorough clinical inspection and palpation; and 2) Detection of high level of leukocytes in milk using the Californian mastitis test (CMT).

**Microbiological procedure:** All positive samples were analyzed microbiologically by CMT and clinical examination according to [14]. Samples from individual quarter were centrifuged at 3000 revolution for 15 minutes and supernatant was discarded. Standard loop full of 0.01ml of milk was then removed from the sediment and cultured on

### 3.1. Questionnaire

3.2. In the farms included in this study no strip cup was used and dry therapy and post milk disinfection were not practiced. Clinically detected cases of mastitis have been treated by parental injection of locally available drug, most of the time by penicillin, streptomycin, and oxytetracyclines. Animal at the Sodd dairy farms and in farmers hand had high tick infestation, despite regular treatment with steladone. As regards nutrition, almost all dairy cows, especially those in peasant's farms, were kept on dry roughage except during rainy season when green feeds are available. Type of housing ranged from open kraals to free stalls in which cow were tied with chains.

### 3.3. Clinical Examination

Of the total 386 cows examined, 134 (34.7%) were found to be positive for mastitis: 52 (38.8%) clinical and 82 (61.1%) sub clinical mastitis. Out of 966 quarters, 212 (21.94%) were found to be affected: 83 (39.15%) clinically and 139 (65.56%) sub clinically affected. In the clinical cases, 12 blood tinged milk with sign of inflammation in the affected quarter, 20 had watery without sign of inflammation in the quarters, 13 milk contained pus with swollen quarter mostly. Seven animals had acute mastitis with signs of inflammation in the quarter and severe inflammation. Among all animal examined, 105 blind quarters were found and those that had an indurate mass due to fibrosis were common in most cows.

### 3.4. Prevalence of Mastitis

Clinical examination and screening test results indicate an overall prevalence rate of 34.7%. The prevalence rate differed

significantly among breeds ( $p < 0.05$ ).

**Table 1.** Prevalence of mastitis by breed in and around Wolaita Soddo.

Breed	No of animal examined	No. of affected	No. of not affected	prevalence
Indigenous zebu	216	50	166	23.1%
Jersey	88	39	49	44.3%
High-grade Holstein	82	45	37	54.8%
Total	386	134	252	34.7%

$\chi^2 = 31.04$ ;  $P = 0.000$

Quarter prevalence of mastitis is found to be 25% in high grade Holstien and 20.82% in indigenous zebu with no significant difference among quarters of the three breeds.

**Table 2.** Quarter prevalence of mastitis by breed.

Breed	No. of quarter examined	Noof affected	No of not affected	prevalence
Indigenous zebu	485	101	384	20.82%
Jersey	281	61	220	21.70%
High grade holstien	200	50	150	25%
Total	966	212	754	21.94%

$\chi^2 = 1.43$ ;  $P = 0.49$

The highest prevalence is seen in early lactation (1-120 days) in which out of 228 animal examined 100 (43.85%) were found positive and the lowest prevalence is seen in end lactation (<240 days) 23%.

**Table 3.** Prevalence of mastitis in different lactation stage.

Stage of lactation	No. of examined	No. of affected	No. of not affected	Total
Beginning (1-120 days)	228	100 (43.85%)	128 (56.14%)	228
Middle (120-240days)	137	44 (32.25%)	93 (68.19%)	137
End (<240 days)	21	5 (23.80%)	16 (76.19%)	21
Total	386	149	237	386

$\chi^2 = 7.03$ ;  $P = 0.000$

The highest prevalence (64.51%) is observed at a parity number 6 and above and the lowest prevalence (26.08%.) observed at first parity number which is the prevalence raised with an increase in parity number ( $r = 0.8$ ).

**Table 4.** Prevalence of mastitis in various parity groups.

Parity number	No. of cowexamined	no. of mastitic animal	None mastitic animal	Prevalence %
1	115	30	85	26.08%
2	93	17	76	39.54%
3	75	29	46	38.66%
4	54	28	26	51.85%
5	18	10	8	55.55%
6 and above	31	20	11	64.51%

The prevalence of 41.66% was seen in animals with lesions and/or tick infestation on teat and /or udder skin as compared to animals without lesions and/or tick infestation, in which the prevalence was 25.88%.

**Table 5.** Prevalence of mastitis in relation to predisposing factors.

Cow	Infected animal	None-infected	Total
With lesion and /or tick on skin or udder	90 (41.66%)	126 (58.33)	216
Without lesion and tick infestation on skin /teat or udder	44 (25.88%)	126 (74.11)	170
Total	134	252	386

$\chi^2 = 12.52$ ;  $P = 0.02$

In the present study, the highest prevalence was seen in housed animals as compared to pastured cows.

**Table 6.** Prevalence of mastitis by management system.

Management	Animals examined	No. of affected	None affected	Prevalence
Pastured	216	50	166	23.14%

Housed	170	84	86	49.41%
Total	386	134	252	34.7%

$\chi^2 = 35.42$ ;  $P = 0.000$

### 3.5. Milk Bacteriology Results

Bacteriological examination was carried out on all CMT and clinically positive udder quarter samples to identify the causative organism involved in the disease. The organisms were identified based on colony morphology, gram stain, hemolysis and biochemical test for species identification. The differentiation of microorganisms was carried out according to [14].

Out of 134 positive animals, 52 were clinically infected while 82 were sub-clinically infected. From 134 positive animals only 111 is culturally positive.

**Table 7.** Prevalence of clinical and sub clinical mastitis.

Infection status	Clinical mastitis	Sub clinical	Total
Infected	52 (38.15%)	82 (61.1%)	134
None-infected	126 (32.64)	126 (32.64%)	252
Total	178	208	386

Of all culturally positive animals, 46 were from clinical and 65 from subclinical cases.

**Table 8.** *Staphylococcus* identification.

hemolysis	Catalase	Tube coagulase	Manitol test	O-F test	Frequency of isolation	Species of bacteria isolated
+ve	+ve	+ve	+ve	F	54	<i>S. aureus</i>
-ve	+ve	+ve	-ve	F	2	<i>S. hyicus</i>
-ve	+ve	-ve	-ve	F	3	<i>S. epidermidis</i>

+ve=positive; -ve=negative; F= fermentation; CMT= Californian mastitis test O-F= oxidation fermentation test; S=staphylococcus

**Table 9.** *Streptococcus* species identification and differentiation results.

Hemolysis	Catalase	CAMP test	Hydrolysis of aesculin on Edward media	frequency of isolation	Species of streptococcus identified
Alpha	-ve	+ve	-ve	10	<i>Strep. agalactiae</i>
Beta	-ve	+ve	-ve	6	<i>Strep. agalactiae</i>
Gamma	-ve	-ve	+ve	7	<i>Strep. dysagalactiae</i>
Beta	-ve	-ve	+ve	4	<i>Strep. dysagalactiae</i>

-ve= negative +ve=positive strep= streptococcus

**Table 10.** *Genera of bacteria isolated from clinical and sub clinical mastitis.*

Cases	Staphylococcus	Streptococcus	Coliform
Clinical	15	7	7
Sub-clinical	25	12	8
Total	40	19	15

Out of all clinical cases, 15 were caused by staphylococcus, 7 were caused by *Streptococcus*, and 7 by coliform species. Out of all subclinical cases, 25 were by *Staphylococcus*, 12 by *Streptococcus* and 8 by coliform species.

## 4. Discussion

The purpose of the research was to find out how common bovine mastitis is in the town of Woliata Soddo in southern Ethiopia. The overall frequency of the illness in cows was 34.7% and in quarters it was 21.94%. This result is in line with the overall claim made by [1], who states that the incidence rate in cows is 40% and in quarters 25% in most nations, regardless of the source. Results from [4] and [15] showing a prevalence of 45.8% in Sudan and 45.5% in Ethiopia, respectively, are in agreement with the infection rate in cows. Compared to the results of [4], which indicated a quarter infection rate of 38.8% in Sudan, this was lower. Factors including the environment, the agent, and the host may contribute to the variance in the general prevalence of mastitis. According to this research, an unclean milking process and ineffective management might lead to a higher infection rate. One possible explanation for the observed variation is that management styles change from one location to another. Zebu, jersey, and high grade Holstein cows had significantly different incidence rates ( $P < 0.05$ ). In contrast to Jersey-holstien crosses, those involving the Holstein Frisian or Haryana breeds were more likely to have subclinical mastitis, according to several writers [16]. In addition, researchers in Kombolcha, Ethiopia, discovered that the prevalence rate of zebu holstien crosses is much greater than that of the zebu breed [18]. The impact of milk output on mastitis incidence may account for this variance. Also, according to [1], high-yielding cows are more likely to be affected. The high milk output stress that may upset the animals' defense system and the ease with which injuries may be incurred in huge udders, which provide foci for the entry of viruses, might be the reasons for this. Due to the lack of interaction between the two breeds during milking, the local zebu, whose prevalence rate was much lower than that of Jersey and Holstein, were at danger of mastitis.

Relative to other breeds, there was a higher incidence of sanitary procedures, lesions, and tick infestations on the surface of the udder and teats.

Prevalence rates of sub-clinical mastitis were 61.19% and clinical mastitis was 38.80% in this research. The yearly incidence rate of 41.2% in England's dairy herds is consistent with the severity of clinical cases. According to studies conducted in Sudan [5] and Ghana [20], there is a 46% difference between sub-clinical and clinical mastitis, whereas in Ghana it is 20% and 41.67%, respectively. Mastitis prevalence was shown to be 43.85% during early lactation (1-120 days), substantially greater than 32.25% during mid-lactation (121-240 days) and 23.25% at the end of lactation, when the influence of lactation stage was evaluated ( $P < 0.05$ ). Consistent with previous research, this data supports the high prevalence seen in early lactation sahiwal cows in India (reference 21).

Multiple forms of physiological stress, including early breastfeeding, may lower immunity and lead to an upsurge in mammary gland infections, as stated in [22]. According to [23], acute mastitis is common during breastfeeding because neutrophils in the mammary glands have a delayed diapedesis, which reduces their resistance. The increased prevalence rate seen in early lactation in this research may be explained by this fact. Animals without this lesion and/or tick infestation on udder and/or teat skin had a prevalence rate of 41.66% compared to animals without these variables at 25.88%. This was done in order to determine the effects of these factors on the prevalence of mastitis. This result was in line with previous reports from [18], [20], [24], and [3]. Staphylococci and, to a lesser degree, streptococcus colonized the teat lesions in this population of animals, which may explain the high occurrence.

Mastitis prevalence was found to be 49.41% in housed cows and 23.14% in pastured cows as a result of the management approach. Because the organisms may be easily transmitted from infected to healthy animals, our results are in agreement with those of [25], who also discovered a much higher incidence rate in housed animals. After conducting a thorough microbiological examination of the sample, it was found that out of the 124 organisms identified, staphylococcus aureus constituted 43.54% of the total.

This finding was consistent with those of [6], [26], all of whom found *Staphylococcus aureus* as predominant isolates from bovine mastitis.

The predominance of *Staphylococcus aureus* could attribute to the wide ecological distribution of the organism on intra mammary and skin of the udder and frequent colonization of the eroded and injured skin on the teat and/or udder of the cows.

[1] The bacterium is able to thrive in the udder and may cause a mild illness that lasts for a long time. It can then spread to healthy animals via milk, which happens mostly when milking is not done properly. Possible causes of the high isolation rate include unsanitary living quarters, insufficient dry treatment, insufficient milking teat dipping, and a low rate of culling for animals who suffer from mastitis. The isolation rate for *Staphylococcus epidermidis* was 2.41% and for *Staphylococcus hyicus* it was 1.61%. The researchers in this investigation identified *S. epidermidis* and *S. hyicus* as coagulase negative *Staphylococcus*.

A recovery rate of 16.12% for *Escherichia coli* was observed; this finding is in agreement with [27], which reported an isolation rate of 11.5%; however, Demelash's reported rate was 3.14%, and *Klebsiella aerogenes* occurred at a rate of 3.23%. The most common source of these coliform organisms in dairy cow environments is manure [3]. According to popular belief, these viruses may live and reproduce in non-mammary areas more effectively than other prevalent mastitis infections. Additionally, no one control approach has been shown to be efficient in laboratory settings. Isolation rates of *Streptococcus agalactiae* were 13%, 10%, and 13.5 percent, respectively, in previous studies [28], [29], and [27]. Although 38% and 37% were lower than the results in [30], they were still below the average. Possible causes for the relatively high isolate of this organism in this research include improper hygiene during milking, not dipping the teats after each milking, not treating animals who were clinically infected, not using dry period therapy, and not culling cows that didn't react to dry period therapy. Among the isolates, 8.87% were *Streptococcus dysgalactiae* and 4% were *Streptococcus uberis*.

Because most of the illnesses were not severe enough to warrant treatment, many of them spread to other cows in the herd and eventually became clinical. While farmers and herd managers may be aware of the symptoms of mastitis, they may not realize how common the illness is or how much money it costs. Antibiotics can help with mastitis control in dairy cows, but they aren't a panacea. What's more, overusing antibiotics like penicillin and streptomycin could lead to the rise of bacteria that are resistant to both drugs. Hence, according to these comments, the following suggestions are made: When it comes to milking, dairy farms should adhere to strict sanitary procedures; Chronically sick cows, whether they have blind quarters or not, should be slaughtered to eliminate a possible vector for disease.

be eradicated; additional research should be conducted, focusing on the factors that put farmers at risk of financial losses due to the disease, in order to raise awareness among farmers about the disease and its impact on dairy production; and short-term training, extension workers, and model farmers should be employed to educate farmers about the disease.

Author Contribution

JSh: conception of the research idea, designing data collection, interpretation of the results and drafting the manuscript. IT: data collection and drafting the manuscript. The authors read and approved the final manuscript.

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