Cross-Sectional Study on Prevalence, Risk Factors and Major Bacterial Causes of Bovine Mastitis in and Around Wolaita Soddo, Southern Ethiopia

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Abstract
A cross-sectional study was conducted from November 2014 to March 2015 on lactating dairy cows to determine the overall prevalence of bovine mastitis, assess concomitant risk factors and isolate the major bacterial agents involved in causing mastitis in and around Wolaita Soddo. A total of 320 lactating cows were examined for mastitis using clinical examination and California mastitis test (CMT). Bacteriological methods were also done to isolate the causative bacteria. An overall 26.25% prevalence of mastitis was recorded in the area of which 12/84 (3.75%) were clinical and 72/84 (22.50%) subclinical cases. About 125 bacterial isolates based on their relative frequency of occurrence were Staphylococcus aureus (39.85%), Coagulase Negative Staphylococcus (CNS) (20.30%), Streptococcus dysgalactiae (13.53%), Streptococcus agalactiae (12.03%), Escherichia coli (8.27%). In this study risk factors like parity, lactation stage, and body condition were shown association with mastitis. Animals with many parity Odds Ratio (OR: 3.55, 95% CI: 1.64 – 7.67, P value: 0.001), moderate (OR: 1.49, 95% CI: 0.76 – 2.91, P: 0.242) were at higher risk than animals with few parity. Animals with early lactation (OR: 3.93, 95% CI: 2.02 -7.66, P: 0.001), mid lactation (OR: 1.63, 95% CI: 0.75 – 0.54, P: 0.214) were at higher risk than animals with late lactation. Animals with poor body condition (OR: 3.33, 95% CI: 1.58 – 7.85, P: 0.002), medium body condition (OR: 1.51, 95% CI: 0.68 – 3.35, P: 0.308) were at risk than animals with good body condition. Generally, the study showed that mastitis was a serious problem for dairy production in the study area. Therefore, appropriate control measures targeting the specific causative agents should be in place to reduce the impact of the disease. The farmers should also be aware of the impact of the disease and practice hygienic milking, culling of chronic mastitis carriers and treating of clinically infected cows.

Keywords: Dairy cattle, Mastitis, Milk, Risk factor, Wolaita Soddo

1. Introduction
Naturally endowed with different agro-ecological zones and suitable environmental conditions, Ethiopia is a home for many livestock species and suitable for livestock production. Ethiopia is believed to have the largest livestock population in Africa (Solomon et al., 2003; Tilahun and Schmidt, 2012; CSA, 2013). An estimate indicates that the country is a home for about 54 million cattle, 25.5 million sheep and 24.06 million goats. From the total cattle population 98.95% are local breeds and the remaining are hybrid and exotic breeds. The livestock subsector has an enormous contribution to Ethiopia’s national economy and livelihoods of many Ethiopians, and still promising to rally round the economic development of the country. Livestock plays vital roles in generating income to farmers, creating job opportunities, ensuring food security, providing services, contributing to asset, social, cultural and environmental values, and sustain livelihoods. The subsector contributes about 16.5% of the national Gross Domestic Product(GDP) and 35.6% of the agricultural GDP (Metaferia et al., 2011). It also contributes 15% of export earnings and 30% of agricultural employment (Behnke, 2010).

The livestock subsector currently supports and sustain livelihoods for 80% of all rural population. The GDP of livestock related activities valued at birr 59 billion (Metaferia et al. 2011). It is important that livestock products and by-products in the form of meat, milk, honey, eggs, cheese, and butter provide mainly the needed animal protein that contributes to the improvement of the nutritional status of the people. Cow represents the biggest portion of cattle population of the country, around 42% of the total cattle heads are milking cows (CSA, 2008; FAO, 2003a).

Diary production is a biologically efficient system that converts feed and roughages to milk (Yohannes, 2003). Milk has high nutritive value rich in carbohydrates, proteins, fats, vitamins and minerals. The total annual milk production of Ethiopia is estimated at 797,900 to 1,197,500 metric tons of raw milk, out of which 85 to 89% is derived from cattle. The per capita milk availability for consumption was estimated at 17 to 18 kg per year compared to the global average of 100 kg per year (FAO, 2003b).

Despite high livestock population and existing favorable environmental conditions, the current livestock output of the country in general and milk production in particularly does not satisfy the country’s requirements due to a multitude of factors. Mastitis is among the various factors contributing to reduced milk production (Biffa et al., 2005).

Mastitis is an inflammation of the mammary gland that can be caused by physical or chemical agents...
but the majority of the causes are infectious and usually caused by bacteria (Quinn et al., 2002). Over 140 different microorganisms have been isolated from bovine intra mammary infection, but the majority of infections are caused by *Staphylococci*, *Streptococci* and *Coliforms* (Radostitis et al., 2007). Mastitis is the most important and expensive disease of dairy industry. It results in severe economic losses from reduced milk production, treatment cost, increased labor, milk withheld following treatment and premature culling (Miller et al., 1993).

In view of the degree of inflammation, mastitis can be classified as clinical and sub clinical types (Philpot and Nickerson, 1991). Clinical mastitis includes gross abnormality in milk, physical abnormalities of udder and abnormality of cow with systemic involvement. Sub clinical mastitis is characterized by absence of gross lesion and an increase in number of somatic cell in the milk. It is more prevalent than clinical mastitis and with long duration, reduced production and affect quality of milk produced (Philpot and Nickerson, 1991). According to Radostits et al., (2007), the diagnosis of bovine mastitis is performed by clinical examination (inspection and palpation) for clinical forms of mastitis, screening or California Mastitis Test (CMT) test for subclinical forms of mastitis and bacterial isolation for confirmatory diagnosis.

In Ethiopia, mastitis has long been known (Bitew et al., 2010), however, the information on the magnitude, risk factors and causative agent of the disease is inadequate. Such information is important when designing appropriate strategies that would help to reduce its prevalence and effects (Biffa et al., 2005). Most studies in Ethiopia were carried out in Addis Ababa and its surroundings, which are not representative of other regions of the country (Almaw et al., 2009). In southern regional state, mastitis is not well considered now a day. There were very few published materials on the current status of mastitis in and around Wolaita Soddo (Tamirat, 2007). Therefore, this study was instigated with the objectives of determining the overall prevalence of bovine mastitis in and around Wolaita Sodo, assessing the concomitant risk factors and isolating the Major bacterial causes.

2. Materials and methods

2.1. Study Area

The study was conducted in and around Wolaita Soddo, Southern Ethiopia. Wolaita Soddo is located about 390 km south of Addis Ababa. The town Soddo is located at latitude of 8°50” N and longitude of 37°45” E. Topographically, the area is marked by hilly, flat, steep slopes and gorges and a number of streams and mountains. The highest mountain is Damota, 2500 m above sea level, which is located near Soddo town (Tamirat, 2007). The Altitude varies from 1100-2950 m.a.s.l. The area experiences mean annual temperature of about 20°C. The mean maximum temperature is 26.2°C and the average monthly minimum temperature is 11.4°C. The rainfall regimes over much of the area are typically bimodal with the big rainy season extending from June to September and a small rainy season occurring from February to April. The mean annual rain falls of the area ranges from 450-1446 mm with the lowest being in low land and highest in high land. The livestock population in the area is estimated to be 886,242 cattle, 117,274 sheep, 99817 goats, 1951 horses, 2174 mules, 54,209 donkeys and 442,428 poultry chickens (Wolaita Zone Agricultural Office, 2013).

2.2. Study Animals and Management Systems

The study populations were all lactating cows in and around Wolaita Soddo. Lactating dairy cattle (local breed) from surrounding areas of Soddo, exotic breeds from Wolaita Soddo dairy farm and private farms in and around Wolaita soddo and cross breeds from the small holder dairy farm in Soddo town constitutes the study animals.

The indigenous zebu cattle found in the study area are managed under extensive system as a source of milk, meat and drought power. The dairy herd of Jersey breed at wolaita Soddo dairy farm is managed semi-intensively under the supervision of trained personnel. Small holder dairy farms in the town are with Holstein-Zebu cross mainly and they are always housed and provided feed in their stall.

2.3. Study Design

A cross-sectional study design was conducted from November 2014 to March 2015 in and around Wolaita Soddo, to estimate prevalence, concomitant risk factors, and to isolate major causative bacteria of bovine mastitis. Cows were examined clinically and tested for mastitis screening with CMT, and cases found clinically mastitis or screening positive were sampled for bacterial isolation. The risk factors considered for the study were, age, parity, lactation stage, breed, hygiene of milking and body condition. Age of the cows was determined by observing their dentition characteristics and grouped into < 5 years (young adult), 5-8 years (adult) and > 8 years (old) category according to Yohannis and Molla, (2013). Parity was categorized into 1-2 calves (few), 3-6 calves (moderate) and > 6 calves (many). Milking hygiene practice was grouped into good (If there is a practice of washing and drying udder with separate towels, milking healthy and young cows first) and poor (If washing and drying of udder with a separate towel and milking with order is not practiced). Lactation stage of the cow was also categorized into early stage lactation (1-4 months), mid stage lactation (> 4 – 8 months) and late stage lactation (above 8 months) (Yohannis and Molla, 2013).
2.4. Sampling Method and Sample Size Determination
A multi stage cluster sampling was used to sample animals. The study area was selected purposively. Lactating cows were sampled randomly from farms and households. Sample size determination was done according to Thrusfield, (2005):

\[ n = \frac{(1.96)^2 \times P \times (1-P)}{d^2} \]

Where \( n \) = sample size, \( P \) = expected prevalence (29.5%), 1.96 = the value of Z of 95% confidence level, \( d = \) Desired absolute precision =5%. For estimation of disease prevalence in the study area a prevalence of 29.5% was taken according to the report of Yohannis and Molla, (2013) and confidence level of 95%, precision level of 5%. Based on the above formula the total required sample size is 320 milking cows.

2.5. Milk Sample Collection
Strict aseptic procedures were adopted when collecting milk samples in order to prevent contamination with microorganisms present on the body of animal and from the barn environment (Quinn et al., 2002). The udder, especially the teats were cleaned and dried before milk sample collection. Then the teats were swabbed with cotton, soaked in 70% alcohol. To prevent recontamination of teats during scrubbing with alcohol, teats on the far side of the udder were scrubbed with alcohol first, then those on the near side (NMC, 1990). Milk sampling and screening were performed for each quarter. The time chosen for sample collection was before milking. Information on the cow age, parity, breed, lactation stage, milking hygiene and body condition were also collected at the time of sampling using data recording sheet (Quinn et al., 2002).

2.6. Diagnosis of Mastitis
In the present study, the lactating cow’s udder and teats were clinically examined by palpation to know the abnormalities before the collection of milk samples. According to Radostits et al., (2007), quarters revealing the following abnormalities were diagnosed as clinical mastitis; observation of abnormal milk with no visible and palpable changes in quarters, observation of abnormal milk with visible and palpable changes in quarters and acute mastitis with systemic involvement. If one of the above symptoms was observed, milk was sampled directly for bacterial isolation. Animals or cases not showing either signs were tested for screening with CMT test; those positive were sampled to perform bacterial isolation.

2.7. Microbiological Procedures
Samples from CMT positive and from clinically mastitic cows were analyzed microbiologically based on Quinn et al., (2002) as absolute diagnosis and identification of the disease was based on isolation and identification of bacteria. Culturing of the collected samples was performed after centrifugation to concentrate the organisms then it was inoculated into blood agar medium. MacConkey agar plates were streaked in parallel to detect gram negative bacteria. Edwards’s media was used as selective media for streptococcal organisms and for determination of hemolysis and aesculin hydrolysis. The inoculated plates were incubated aerobically for 24 to 48 hour at 37°C. The result was declared as negative, if growth did not occur after 72 hour of incubation.

Bacterial isolates were identified on the basis of colony characteristics, presence of hemolysis, catalase test, KOH test, Gram’s stain and biochemical tests. The biochemical tests which employed in this study were: Coagulase test (Quinn et al., 2002) was employed to differentiate Staphylococcus aureus from other Staphylococcus species, Aesculin hydrolysis on Edwards medium, Christie, Atkins, and Munch-Petersen test (CAMP) test were also used to differentiate Streptococcus agalactiae from other mastitis causing Streptococci and Triple sugar iron (TSI) test and Indole Methyl red Voges-proskauer Citrate utilization (IMVIC) test were carried out in the study to differentiate gram negative coliforms such as E.coli, Salmonella etc.

2.8. Statistical Analysis
Raw data were first entered into a Microsoft Excel spreadsheet and analyzed using STATA 11 software. Descriptive statistical analysis was used to summarize and present the data collected. The prevalence of mastitis was calculated as the number of lactating cows tested positive by CMT test or animals showing symptoms of clinical mastitis, divided by the total number of tested or clinically examined animals. The existence of association between the risk factors (age, parity, lactation stage and body condition) and mastitis was assessed using the Pearson Chi-square (χ²) test. Besides, the degree of association between the risk factors and occurrence of mastitis were analyzed first with univariate logistic regression and those factors having a P-value less than 0.25 were further considered in multiple logistic regression analysis in the final model. Significant values were considered at P<0.05.
3. Result

3.1. Overall Prevalence of Bovine Mastitis

From the total of 320 lactating cows examined, 84 (26.25%, 95% CI: 21.4 – 31.1) were positive for mastitis. Of these, 12 (3.75%) and 72 (22.50%) were found to be positive for clinical mastitis and subclinical mastitis, respectively. The results of bacterial cultures also revealed that 76 (90.48%) of the affected cows were with bacterial isolates and the rest 8 (9.52%) were without bacterial isolates. The study also considered mastitis at quarter level. Of 1280 quarters examined, 133 (10.39%) were found to be CMT positive in case of subclinical mastitis and mastitis positive in case of clinical mastitis and 76 (5.94%) quarters were blind.

3.2. Risk Factors

Among the eight potential risk factors considered for a univariate logistic regression, most of the risk factors such as age, parity, lactation stage and body condition were found significantly associated with mastitis (P<0.05) (Table 1).

Table 1. Univariate logistic regression analysis of risk factors for the occurrence of bovine mastitis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Total examined (+ve)</th>
<th>Prevalence</th>
<th>Chi-square</th>
<th>P-value</th>
<th>OR(CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Residence</td>
<td>Peri-urban</td>
<td>172(47)</td>
<td>27.33</td>
<td>0.22</td>
<td>0.64</td>
<td>1.13 (0.68-1.68)</td>
<td>0.637</td>
</tr>
<tr>
<td></td>
<td>Urban</td>
<td>148(37)</td>
<td>25.00</td>
<td>0.11</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Management</td>
<td>Intensive and semi-inten.</td>
<td>35(10)</td>
<td>28.57</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extensive</td>
<td>285(74)</td>
<td>25.96</td>
<td>1.14</td>
<td>0.74</td>
<td>0.52-2.48</td>
<td>0.741</td>
</tr>
<tr>
<td></td>
<td>Young adult</td>
<td>32(6)</td>
<td>18.75</td>
<td>1.13</td>
<td>0.74</td>
<td>0.52-2.48</td>
<td>0.741</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>162(35)</td>
<td>21.60</td>
<td>0.11</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>126(43)</td>
<td>34.13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hygiene</td>
<td>Good</td>
<td>138(31)</td>
<td>22.46</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>182(53)</td>
<td>29.12</td>
<td>1.42</td>
<td>0.74</td>
<td>0.52-2.48</td>
<td>0.741</td>
</tr>
<tr>
<td>Parity</td>
<td>Few</td>
<td>79(14)</td>
<td>17.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>181(44)</td>
<td>24.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Many</td>
<td>60(26)</td>
<td>43.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactation</td>
<td>Late</td>
<td>113(17)</td>
<td>15.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>stage</td>
<td>Mid</td>
<td>79(18)</td>
<td>22.78</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Early</td>
<td>128(49)</td>
<td>38.28</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breed</td>
<td>Local</td>
<td>193(46)</td>
<td>23.83</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cross</td>
<td>37(12)</td>
<td>32.432</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exotic</td>
<td>90(26)</td>
<td>8.88</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body</td>
<td>Good</td>
<td>77(11)</td>
<td>14.28</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>condition</td>
<td>Medium</td>
<td>139(32)</td>
<td>23.02</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>104(41)</td>
<td>39.42</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2 Multivariate logistic regression of risk factors for the occurrence of Mastitis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>OR</th>
<th>CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td>Moderate</td>
<td>0.96</td>
<td>0.38-2.43</td>
<td>0.927</td>
</tr>
<tr>
<td></td>
<td>Many</td>
<td>1.95</td>
<td>0.57-6.70</td>
<td>0.287</td>
</tr>
<tr>
<td>Lactation</td>
<td>Mid</td>
<td>1.63</td>
<td>0.75-0.54</td>
<td>0.214</td>
</tr>
<tr>
<td></td>
<td>Early</td>
<td>3.93</td>
<td>2.02-7.66</td>
<td>0.001</td>
</tr>
<tr>
<td>Body condition</td>
<td>Medium</td>
<td>1.51</td>
<td>0.68-3.35</td>
<td>0.308</td>
</tr>
<tr>
<td></td>
<td>Poor</td>
<td>3.53</td>
<td>1.58-7.85</td>
<td>0.002</td>
</tr>
<tr>
<td>Age</td>
<td>Adult</td>
<td>1.12</td>
<td>0.33-3.84</td>
<td>0.852</td>
</tr>
<tr>
<td></td>
<td>Old</td>
<td>1.33</td>
<td>0.31-5.60</td>
<td>0.699</td>
</tr>
<tr>
<td>Hygiene</td>
<td>Poor</td>
<td>1.59</td>
<td>0.91-2.78</td>
<td>0.102</td>
</tr>
</tbody>
</table>

3.3. Bacterial Isolation

Analysis of bacteriological examination of milk samples were made to identify the main etiological agents involved in the disease. The organisms were identified on the basis of their cultural, staining characteristics and biochemical reactions. In the study period, about 5 bacterial species and 125 bacterial isolates: *S. aureus*, 53 (39.85%), *CNS*, 27 (20.30%), *Str. dysagalactiae*, 18 (13.53%), *Str. agalactiae*, 16 (12.03%), *E. coli*, 11 (8.27%),
and the isolates with no growth about 8 (6.02%) were observed (Table 3).

### Table 3 The frequency of bacteria isolated from bovine mastitis in and around Wolaita Soddo, Southern Ethiopia

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Clinical mastitis</th>
<th>Sub-clinical mastitis</th>
<th>Total</th>
<th>Proportion %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>10</td>
<td>43</td>
<td>53</td>
<td>39.85%</td>
</tr>
<tr>
<td>CNS</td>
<td>-</td>
<td>27</td>
<td>27</td>
<td>20.30%</td>
</tr>
<tr>
<td><em>Str. dysgalactiae</em></td>
<td>10</td>
<td>8</td>
<td>18</td>
<td>13.53%</td>
</tr>
<tr>
<td><em>Str. agalactiae</em></td>
<td>1</td>
<td>15</td>
<td>16</td>
<td>12.03%</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>4</td>
<td>7</td>
<td>11</td>
<td>8.27%</td>
</tr>
<tr>
<td>No growth at all</td>
<td>-</td>
<td>8</td>
<td>8</td>
<td>6.02%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>25</strong></td>
<td><strong>108</strong></td>
<td><strong>133</strong></td>
<td><strong>100%</strong></td>
</tr>
</tbody>
</table>

4. Discussion

The result revealed an overall prevalence of 26.25% in the study area. The prevalence recorded in this study line with the report of (Girma et al., 2012), who recorded an overall prevalence of 23.18% at West Harergho zone. Current result agrees with the previous report by Bitew et al., (2010), who recorded an overall prevalence of 28.2% at Bahir Dar and its surroundings. The current finding is slightly lower than that of Biffa et al., (2005), who reported an overall prevalence of 37.2% in the urban and peri-urban dairy farms at Addis Ababa, central Ethiopia and 34.9% in the southern Ethiopia. The present study reveals comparably very low when compared with the work of Sori et al. (2005) and Lakew et al. (2009), who reported a prevalence of 52.78% in and around Sebeta and 64.4% in Asella, respectively. Current finding on prevalence of mastitis is also lower than that of Rahman et al., (2010), who reported 53.30% prevalence in dairy cows in Bangladesh. Mastitis is a complex disease and the difference in results could be due to difference in management system of the farm, variation in the susceptibility of different breeds of cattle to mastitis causing organisms and the difference in geographical locations of the studies (Yohannis and Molla, 2013).

The prevalence of clinical mastitis recorded in the present study is 3.75% and that of sub-clinical mastitis is 22.50%. This finding is in line with the report of Bitew et al., (2010), who reported clinical prevalence of 3% and also subclinical cases of 25.2% at Bahir Dar and its surroundings. However, it is lower than that from the findings of Nesru, (1999), who recorded 5% clinical and 32.2% sub clinical cases in the urban and peri-urban dairy farms at Addis Ababa, central Ethiopia. The results of both clinical and subclinical mastitis in this study are also incomparable with those previous reports in Ethiopia (Lakew et al., 2009). In most reports including the present study, clinical mastitis is far lower than subclinical mastitis (Haftu et al., 2012). This could be attributed to little attention given to subclinical mastitis, as the infected animal shows no obvious symptoms and secrets apparently normal milk, especially small holders, are not well informed about invisible loss from sub clinical mastitis. In Ethiopia, the subclinical forms of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases (Almaw et al., 2008).

The overall quarter level prevalence recorded in this study (39.58%) is lower than that of Mekibir et al., (2010), who reported 44.80% overall prevalence at Holota town Central Ethiopia. In comparison with the Nesru et al., (1999) and Biffa et al., (2005) who reported, 37% and 28.20% respectively the present study is slightly higher. The current result was strongly higher compared to Girma et al., (2012), who reported 8.03% overall prevalence in West Harergho zone. From the 133 CMT positive quarter milk samples, 125 (93.98%) were bacteriologically positive up on culturing, while 8 (6.02%) were bacteriologically negative, which lower than the result of Aregaw, (1992), who reported 18% bacteriologically negative samples. The failure of isolation of bacterial agents from CMT positive quarter milk is due to the predominance of subclinical mastitis in the area. Since the animals studied were mainly found in the rural area where veterinary services are not adequate, most farmers use treatment on their own or get the services from Para veterinarians. Lack of stringency in provision of therapy can possibly change the clinical cases into subclinical mastitis. The administration of antibiotics can suppress the bacterial agents and inhibit their growth in the media. It could also be due to spontaneous elimination of infection, intermittent shedding of pathogens, intracellular location of pathogen and the presence of inhibitory substances in milk (Radostitis et al., 2007). It might also be due to some cases of delayed healing of infection from which organism may disappear or reduced, while infiltration of leukocytes continued until healing is completed (Sori et al., 2005). Sampling only positive quarters may underestimate the prevalence of subclinical mastitis; however, this can be taken as a limitation of this study.

The result of current study revealed that age has statistically significant association with mastitis ($x^2 = 6.77, P \text{ value} = 0.034$). The occurrence of more cases of mastitis in older animals observed in the present study is in agreement with reports of Biffa et al., (2005) who also found strong association between age and prevalence of mastitis. This could be due to biological difference of animals, i.e. as the age increased the probability of getting mastitis will be increased.

The current study revealed the significant association of mastitis occurrence with parity. The likelihood of getting mastitis at parity of greater than 4 is 3.55 times (OR: 3.55, 95% CI: 1.64-7.67, P value: 0.001) and at
3-4 is 1.49 times (OR: 1.49, 95% CI: 0.76-2.91, P value: 0.242) as compared to 1-2 parity level. According to Erskine (2001) 1-2 parity has more effective defense mechanism than parity greater than 4. The increased prevalence of mastitis with parity reported in the current study is comparable with the previous reports (Mekibib et al., 2010; Haftu et al., 2012). 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 (Radostits et al., 2007).

In the present study there is statistical significant association between mastitis and stage of lactation. The likelihood of getting mastitis in early lactation is 3.5 times (OR: 3.5, CI: 1.87-6.55, P value: 0.001) and in mid lactation is 1.67 times higher (OR: 1.67, CI: 0.79-3.48 P value: 0.174) as compared to late lactation. The early lactation stage infection might be due to the carryover of infection from dry period. In cows most new infections occur during the early part of the dry period and in the first two months of lactation (Radostits et al., 2007). That is, higher infection in cows in early lactation stage followed by medium and late lactation stages, that concurs with previous reports of (Biffa et al., 2005; Tamirat, 2007).

As the present study reveals the prevalence of mastitis was shown that there is statistical significant association with body condition of the animal. The likelihood of getting mastitis with poor body condition is 3.90 times higher (OR: 3.90, CI: 1.84-8.26, P value: 0.001) and with medium body condition 1.79 times (OR: 1.79, CI: 0.85-3.80 P value 0.127) as compared to good body condition. This could probably associate with the ability of the immune system of an animal to defend infection causing agents (Fufa et al, 2013).

In the current study, five bacterial species were isolated: S. aureus, CNS, Str. dysagalactiae, Str. agalactiae, and E. coli. This report closely agrees with the reports of Sori et al., (2005), Lakew et al., (2009) and Bitew et al., (2010). Bacterial isolates were recorded from all 12 clinically affected cows. The isolates from these clinical cases were S. aureus, Str. dysagalactiae and E. coli; this is in agreement with Mekibib et al., (2010), who reported all the clinical cases which were culture positive and the same species of bacteria at Holeta town, central Ethiopia.

S. aureus was the predominant pathogen involved in constituting 39.85% of all bacterial isolates in the current study. This concurs with Delelesse (2010) but not with Atyabi et al., (2006) or Mekibib et al., (2010) which were lower and higher, respectively than the current study. The relative high prevalence of S. aureus in the current study shows the absence of dry cow therapy and low culling rate of chronically infected animals practice in the study area. There are also other coagulase negative staphylococci bacteria like S. epidermids which contributes about 20.30% of the isolates which is higher than the report of Sori et al., (2005) in and around Sebeta (14.93%).

Streptococcus species were also found prevalent with 25.56% share of the total isolates: Str. agalactiae 12.03% and Str. dysagalactiae 13.53%. This finding coincides with that of Hawari and Aldabbas (2008), who reported 26.2% relative frequency of Streptococcus species in Jordan. However, the finding is higher than the reports of Bitew et al., (2010) at Bahir Dar and its environs (13.9%) and Sori et al., (2005) in and around Sebeta (3.73%) and lower than the report of Atyabi et al., (2006) at farms around Tehran (33.54%).

E. coli is occurred in moderate extent by contributing 17.8% of the isolates. This finding is different from the previous reports by Mekibib et al., (2010) at Holeta (4.6%) and Sori et al., (2005) in and around Sebeta (0.75%), but agrees with the report of Hawari and Aldabbas (2008) in Jordan (15.6%). E. coli is an environmental contaminant and its high prevalence in the pre-sent report could be related to hygienic status practiced at the study site, particularly at Wolaita Soddo dairy farm where the milking hygiene practice is very poor.

There are also other coagulase negative bacteria like S. epidermids which contributes about 20.30% of the isolates which is higher with the report of Sori et al., (2005) in and around Sebeta (14.93%).

5. Conclusions and recommendations
The present study showed high prevalence of the disease which has a significant impact in the production of cattle. The occurrence of the disease significantly associated with concerned risk factors, such as age, parity, lactation stage and body condition. Also this study revealed considerable prevalence of mastitis with the isolation of major pathogens such as S. aureus, CNS, Str. dysagalactiae, Str. Agalactiae and E. coli in the study area. The isolated pathogens are contagious, environmental and teat skin opportunistic. Thus, it requires good attention and management practices to control or prevent the occurrence of the disease. The proper isolation and identification of the causative organism plays a significant role in the prevention and control of the disease. Therefore, based on the above conclusions the following recommendations are forwarded

✓ Further and detailed epidemiological studies should be conducted to determine the prevalence of the disease at regional and national levels.
✓ Appropriate control measures targeting the specific causative agents should be in place to reduce the impact of the disease on dairy industry of the study area.
✓ The farmers should also be aware of the impact of the disease and practice hygienic milking, culling of chronic mastitis carriers and treating of clinically infected cows.
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7. REFERENCES


