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# Prevalence and Identification of Bacterial Pathogens Causing Bovine Mastitis from Crossbred of Dairy Cows in North Showa Zone of Ethiopia

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**Abstract:** A cross-sectional study was conducted from January to May 2012 on 144 lactating crossbred dairy cows in selected districts of North Showa Zone of Ethiopia and aimed to determine the prevalence and risk factors of bovine mastitis and identifying bacterial pathogens associated with the disease. The overall prevalence of mastitis was 128 (88.9%), out of which 12 (8.3%) clinical and 116 (80.6%) were subclinical cases. Of 576 quarters examined, 5(0.87 %) were blind teats and 337(58.5%) quarters showed evidence of infection of mastitis. High score of California Mastitis Test (CMT) positive milk sample collected and investigated microbiologically and many bacterial species were identified. The major isolated bacteria include: Coagulase negative *Staphylococcus* (CNS) 20(40%), *Staphylococcus aureus* 17(34%), *Streptococcus uberis* 1(2%), *Corynebacterium bovis* 1(2%), *C. pseudotuberculosis* 3(6%), *Diplococcus* spp. 2(4%), *Micrococcus* spp. 1(2%), *Bacilus* spp. 1(2*%), E. coli* 3(6%) and *Pseudomonas* spp. 1(2%). The Chi-square ($X^2$) analysis revealed that there was a significant differences ($P<0.05$) between lactating cows at different lactation stages; however, there was no significant differences ($P>0.05$) between lactating cows with parity, hygiene and husbandry conditions. The present study showed that mastitis is the problem of dairy cows in the study area and the major isolates were contagious pathogens therefore, hygienic milking practice, culling of chronically infected cows and hygienic practices are important tools to control mastitis in the study area.

**Key words:** Bacterial Pathogens · Bovine Mastitis · Cross-Sectional · Crossbred Cows · Prevalence

## INTRODUCTION

Ethiopia constitutes the largest livestock population in Africa with different distribution and quantities depending on animal population system and agro-ecological zone [1]. From those cows represent the highest proportion and 42% of total cattle heads for the private holdings are milking cows even though the rich potential from the livestock sector of the country is not efficiently used due to several constraints including malnutrition, traditional management and disease [2]. Bovine mastitis is the second most frequent disease next to reproductive disorders and one of the major causes for economy failure in Ethiopia. It affects both the quantity and quality of milk [4]. About 140 to 200 USD /Cow/Year is lost due to mastitis with approximately 8% being due to discarded milk, 8% for treatment cost, 14% to death and premature culling and 70% to reduced milk production [5].

Mastitis induced via pathogenic microorganisms that generally come from two sources: the environment (*E. coli*, *Entrobacter* and *Klebsiela* that acquired exposure of teat to contaminated environment, or the animal itself (*Staphylococcus aureus* and *Streptococcus agalactiae*) that comprise contagious bacteria causing mastitis [6] Coagulase negative *Staphylococcus* (CNS) species, *Micrcoccus* species, *Corynebacterium* species, *Bacillus* species, *Pasteurella* species, *Mycoplasma* species and *Nocardia* species are isolated from collected milk samples [7]. *Streptococcus agalactiae*, other *Streptococcus* species, *Staphylococcus aureus* and CNS are predominant pathogens to cause bovine mastitis [8].

In Ethiopia mastitis prevalence rate was 85.6% and 81.2% using California Mastitis Test (CMT) and somatic cell count (SCC), respectively [9]. An overall prevalence of 30.2 and 5.5% for subclinical and clinical respectively in a study conducted in urban and per-urban dairy

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production system in and around Addis Ababa [10]. In addition, 43 and 75% prevalence of bovine mastitis were reported in different parts of Ethiopia [11, 12]. The total cattle population of Oromia Regional State and North Showa Zone is estimated to be 18,035,686 and 1,173,543, respectively [1]. Despite high cattle population and milk production potential of the study area, bovine mastitis is insufficiently investigated and information relating to its magnitude, distribution and risk factors is scant. Thus, the objectives of the study were to determine the prevalence of clinical and subclinical mastitis at cow and quarter level in small holder crossbred dairy cows by identifying major bacterial pathogens that are associated with the disease and to assess the major risk factors associated with bovine mastitis in the study area.

## MATERIALS AND METHODS

**Study Area and Period:** The study was conducted in three districts (Girar Jarso, Wuchale and Debre Libanose) of North Showa Zone of Ethiopia from January to May 2012. They are located at about (Girar Jarso 120 Km, Debre Libanos 106 Km and Wuchale 80 Km) North West of Addis Ababa. Geographically, the zone is situated between 9°-10°24 North latitude and 37°57-39°33 East latitude with elevation of 2633-2835 m a.s.l. The total area coverage of the zone is 11, 607 $km^2$ and daily mean maximum and minimum temperature is 20.98°C and 11.76°C, respectively. The mean annual rainfall of the zone is 1026 mm [13].

**Study Population:** Study animals in the area were all lactating crossbred dairy cows found in the three districts, which were selected purposively based on number of crossbred cows, potential milk production capacity and accessibility. The cows were managed under small scale extensive, semi-intensive and intensive management systems. The animals were supplemented with unbalanced diet and they were feed on natural posture, hay and agricultural byproducts. Intensively managed animals were provided with hay whereas semi-intensive managed animals feed on natural pasture and hay. Extensive managed animals rely on natural pasture.

**Sampling Method and Sample Determination:** Simple random sampling technique was used in order to constitute crossbreed dairy cows in the area were employed. The total sample size was calculated based predetermination of the following parameters: a 95% level confidence, 5% desired level of precision and 89.54% (estimated prevalence from previous report of Kifle and Tadele [14]. A total of 144 lactating crossbreed cows were sampled based on the formula given by Thrusfield [15].

**Study Design and Methodology:** Determination of prevalence, assessment of associated risk factors and identification of major bacterial causing bovine mastitis were determined using a cross-sectional study at cow and quarter level by using clinical manifestation for clinical mastitis and CMT for subclinical mastitis. A well-structured questionnaire was developed and each small holder's dairy farmers in the study area were interviewed with the objective of obtaining information about bovine mastitis and all information relating to the study objectives was recorded. The collected data includes type of dairy husbandry system, parity number, lactation stage and hygiene. Physical examination was performed by inspection and palpation of the mammary glands and collecting and inspection of milk sample. The gland/udder was observed for the clinical signs of mastitis such as swelling, hotness, pain reaction and hardness and the milk sample was observed for the presence of pus, clot, blood and watery consistency.

Milk samples were collected aseptically as procedure described by Quinn and his colleagues [16]. The milk sample was collected before milking. Udder and teats were cleaned using tap water and after drying, each teat was rubbed vigorously with 70% ethyl alcohol socked cotton. In all cases separate cotton was used for each teat. The first two streams of milk were discarded and the milk sample was collected into a sterile test tube and labeled by water proofed marker. The collected sample was then placed in icebox and then transported to Asella regional veterinary laboratory and the CMT was performed from January 19 to May 27, 2012.

Milk samples were bacteriologically examined according to procedure described by Quinn [17]. In refrigerated milk samples bacteria may be concentrated in the clean layer and held with in clumps of fat globule [18]. Hence dispersion of fat and bacteria was accomplished by warming the sample at 25°C (room temperature) for 15 minutes and shacked before plating on a standard bacteriological media. A loop full of milk sample collected from each infected quarter was inoculated separately on to nutrient and blood agar. The inoculated plates were incubated aerobically for 24-48 h at 37°c. The plate was examined for growth, morphology features (colony size, shape and color) and hemolytic activity. Suspected colonies were sub-cultured on

a new plate for further investigation. For primary identification of bacteria, once a pure culture is obtained, a Gram-stained smear from the culture was used to categorize the bacteria based on Gram-reaction and cellular morphology. Then Biochemical tests such as catalase, oxides and triple sugar iron (TSI) test and coagulase tests were used to identify the bacterium to its genus level and maltose, sucrose, lactose, glucose, trehalose, sorbitol, inulin, raffinose and salicin tests were performed to identify the bacterium to its species level [19].

**Data Analysis:** All collected data were entered to Micro-Soft Excel sheet and analyzed by SPSS version 17. Descriptive statistics was used to determine the prevalence of bovine mastitis depending on clinical inspection and CMT and Chi-square test was employed to see the impact of different risk factors on the occurrence of bovine mastitis.

## RESULTS

Of the total 144 lactating cows examined overall mastitis prevalence in the area was 128 (88.9%). The results showed that the prevalence of clinical and subclinical mastitis were 8.3% and 80.6% respectively (Table 1). The result showed that bovine mastitis in Girar Jarso district was higher as compared to the other districts of study area (Table 2). Out of 576 quarters examined 5 (0.86%) quarters were blind, leaving 571 functional quarters. The prevalence of mastitis on quarter basis was 337 (58.5%). The result showed that higher infection rate in back quarters as compared to the front quarters (Table 3). The result showed that the effect of lactation stage was statistically significant (P<0.05) on the prevalence of bovine mastitis and the infection rate is high in animals in early (97.8%) and late (85.1%) lactation stage as compared to the mid lactation stage (83.9%). Animals managed in semi-intensive and intensive husbandry practice showed high rate of infection 92.2% and 88.0% respectively, than managed extensively (76.5%). The infection rate is lower in animals giving one

Table 1: The overall prevalence of bovine mastitis

| Mastitis condition | No. cows Examined | No. of positive (%) |
|---|---|---|
| Clinical mastitis | 144 | 12 (8.3) |
| Subclinical | 144 | 116 (80.6) |

Table 2: Prevalence of bovine mastitis in the three districts.

| Districts | No. of animals | No. of positive (%) |
|---|---|---|
| Girar Jarso | 54 | 52(96.29) |
| Libanose | 49 | 47(95.91) |
| Wuchale | 41 | 28(68.29) |
| Total | 144 | 128(88.9) |

Table 3: The prevalence of mastitis at quarter level.

| Quarter | No. of quarters examined | No. of mastitis positive (%) | No. of blind (%) teat |
|---|---|---|---|
| Right front | 144 | 84 (58.33) | 2 (0.34) |
| Right back | 144 | 90 (62.5) | 2 (0.34) |
| Left front | 144 | 82 (56.94) | 0 (00) |
| Left back | 144 | 86 (59.72) | 1 (0.17) |
| Total | 576 | 337 (58.5) | 5 (0.86) |

Table 4: Prevalence of bovine mastitis based on lactation stage, husbandry practice and hygiene and parity number

| Risk factors | | No. of cows examined | No. of positive (%) | $X^2$ | P-value |
|---|---|---|---|---|---|
| Lactation Stage | Early | 46 | 45 (97.8) | | |
| | Mid | 31 | 26 (83.9) | 5.04 | 0.025 |
| | Late | 67 | 57 (85.1) | | |
| Husbandry | Extensive | 17 | 13(76.5) | | |
| | Semi-intensive | 77 | 71(92.2) | 3.55 | 0.169 |
| | Intensive | 67 | 44(88.0) | | |
| Hygiene | Good | 61 | 54(88.5) | | |
| | Bad | 83 | 74(89.2) | 0.014 | 0.905 |
| | Good | 61 | 54(88.5) | | |
| Parity | 1 | 44 | 36(81.8) | | |
| | 2 | 37 | 34(91.9) | 3.40 | 0.182 |
| | =3 | 63 | 58(92.1) | | |

$X^2$ expressed at df=2, P<0.05 statistically significant

Table 5: Identified bacteria and their relative prevalence

| Species of bacteria | Frequency of occurrence | Percentage (%) |
|---|---|---|
| *S. aureus* | 17 | 36.96 |
| CNS | 20 | 43.47 |
| *Diplococcus* spp. | 2 | 4.34 |
| *C. pseudotuberculosis* | 3 | 6.52 |
| *C. bovis* | 1 | 2.17 |
| *S. uberis* | 1 | 2.17 |
| *Micrococcus* spp. | 1 | 2.17 |
| *Pseudomonas* spp. | 1 | 2.17 |
| *Bacillus* spp. | 1 | 2.17 |

birth (81.8%) than animals with 2 and >3 parity status. Parity, husbandry and hygiene show no statistically significant (P>0.05) effect on the prevalence of bovine mastitis (Table 4). From the CMT high score (2+ and 3+) 60 milk samples were cultured and 50 (%) bacteria were grown on blood and nutrient agar aerobically and CNS, *S. aureus* and *C. pseudotuberculosis* were the most prevalent in the area causing bovine mastitis and the other such as *Diplococcus, C. bovis, S. uberis, Micrococcus, Bacillus, E. coli* and *Pseudomonas* are rarely isolated (Table 5).

## DISCUSSION

This study showed the overall prevalence of mastitis in smallholder crossbreed dairy cows in north Shawa Zone to be 88.9%, which is higher than most of the previous reports in Ethiopia and it is in agreement with the bovine mastitis reported by Kifle and Tadele [18] with the prevalence of bovine mastitis 89.5% in the same area. This finding is also comparable with the findings of Nesru [20], who reported 85.6% bovine mastitis prevalence around Sebeta, Ethiopia. However, the result of the present finding is far higher than (in decreasing order) the previous findings of Tariku *et al*. [21], Mekibib *et al*. [22] Zerihun [23], Lakew *et al*. [24], Bedada and Hiko [25], Bedacha *et al*. [26], Tola [27] and Bedane *et al*. [28], Biru [29] and Demelash *et al*. [11], who reported 75.2, 71.3, 68.1, 65.6, 66.1, 61.11, 59.1, 56.5, 43.5 and 36% bovine mastitis prevalence in different areas of Ethiopia, respectively. The present study showed high prevalence of mastitis in Girar Jarso (96.29%) and (95.91%) districts as compared to the Wuchale district (68.29%). The difference in the prevalence of bovine mastitis among the reports and districts could be attributed to difference in management of the farms, breeds considered, or technical know-how of the investigators.

From 576 examined quarters 337(58.5%) were CMT positive and 5(0.87%) quarters were blind. This finding closely related with the finding of Kifle and Tadele [14] and Birhanu [30], who reported 63.1 and 52.4%, respectively at quarter level. Mekibib *et al*. [22] reported that the prevalence of blind teat was 14% which is far higher than the present finding. Clinical mastitis prevalence at cow level was 8.3% and this is little higher than the report of Husien *et al*. [9] and Bishi [10], who reported 5.7 and 5.3%, respectively in different parts of Ethiopia. However the result of the present study was lower than the findings of Bitew *et al*. [31], Bedada and Hiko [25] and Demelash *et al*. [11], who report 10.3, 10 and 11.9% respectively in different parts of Ethiopia. This may due to concurrent disease involvement, interaction of several risk factors relating with animal and virulence of causative organism.

The prevalence of clinical type of bovine mastitis (8.3%) is lower than the previous findings of Bedada and Hiko [29] and Workineh *et al*. [7], who reported the prevalence rate of 10.3 and 21.5% respectively, in different parts of Ethiopia. However it is greater than prevalence of clinical type of bovine mastitis (4.95%) small holder lactating cows studied at Hawassa, Ethiopia [32]. The prevalence of sub clinical mastitis of this finding reveal 80.6%, which is somewhat comparable with the prevalence (89.5%) reported by Argaw and Tolosa [33]. However, the prevalence of sub clinical mastitis in present study is far higher than previous reports of Bishi [10], Ahmed *et al*. [34], Moges *et al*. [32] and Mekibib *et al*. [22], who studied its prevalence 34.4, 36.67, 30.6 and 25.22% respectively, in different areas of Ethiopia. This could be due to improper milking hygiene, lack of post milking teat dipping and poor housing facilities. Lactation stage shows statistically significant effect on the occurrence of mastitis (P <0.05) which is similar with the report of Biru [29] and those at early and late stage of lactation are at a great risk to mastitis than mid stage of lactation. This could be due to the delayed diapedesis of neutrophils to mammary gland in recently calved cow and at late lactation there is decrement of neutrophil concentration when the cows reach to dry off [7]. Unlike lactation stage; husbandry, parity and hygiene show statistically insignificant effect on the prevalence of mastitis (P >0.05). This could be due to similar farming system, managemental practice and mastitis could be attributed to infectious causes rather a physiology effect.

From the isolated bacteria the most dominant pathogenic species in the study area were CNS (43.47%) followed by *S. aureus* (36.95%) that were the predominant causes of clinical and subclinical mastitis in the area. This finding is comparable with the finding of Bitew *et al*. [31] who reported CNS (51.9%). In addition, the present finding somewhat in agreement with the reports of Sori *et al*. [21 and Abdel-Rady *et al*. [34] who reported the prevalence of mastitis caused by *Staphylococcus* spp.

as 39.44 and 41.4% respectively. However, Mekibib *et al*. [22] reported the occurrence of *S. aureus* as 47.1%, which is a little higher occurrence and greatly lower than Workineh *et al*. [7] who reported 70.5% bovine mastitis cases caused by *Staphylococcus* spp. This difference could be due to lack of effective udder washing and drying, inter-cow hand washing and disinfection in the area. The relative lower prevalence of mastitis caused by *C. pseudotuberculosis* in this study (6.52%) in agreement with reports of Tolla (6.66%) [27], Biffa (4.55%) [35] and Biru (4.2%) [29], but far lower than the report of Sori *et al.* (14.18%) [36]. The isolation of *Diplococcus* spp. in low prevalence (4.34%) in this study compared with the isolation similar pathogen in dromedary (*Camelus dromedariu*s) in the United Arab Emirates by Quandil and Oudar [37].

The rarely isolated bacteria in this finding were *C. bovis, S. uberis*, *Bacilus* spp., *Micrococcus* spp. and *Pseudomonas* spp., in agreement with the reports of Tariku *et al*. [21], Biffa [35] and Sori *et al.* [36]. Similarly, *B. cereus* and *Bacillus spp.* bacteria are rarely isolated organisms that are saprophytic in causing mastitis in dairy animals [6, 37]. The hind quarter have high mastitis prevalence and it could be due to greater production capacity of hind quarter, likelihood of fecal accumulation environmental contamination and difficulty of cleaning [38].

## CONCLUSION

The study was designed to determine the prevalence of bovine mastitis in lactating crossbred dairy cows where clinical and subclinical mastitis differentiated using clinical sign, history, CMT. In addition, isolation and identification of major mastitis causing bacteria and associated risk factor were included in the study. The overall prevalence is so high from most of previous study in different part of Ethiopia. Farmers and herd manager are only concerned with clinical form of mastitis and often are unawareness of the status of subclinical infection in the herd. The major isolated bacteria were CNS, *S. aureus, C. pseudotuberculosis*, *C. bovis, S. uberis*, *Micrococcus* spp., *Diplococus* spp., *Bacilus* spp. and *Pseudomonas* spp. Based on the findings the authors suggests that chronically ill and old aged animals should be culled to eliminate the potential source of pathogens for the occurrence of mastitis and periodic monitoring of infection status of the udder should be under taken in order to control mastitis in the study area.

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