

*Full Length Research Paper*

## Study on bovine mastitis and associated risk factors in Adigrat, Northern Ethiopia

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A cross sectional study was conducted from November, 2011 to April, 2012 in Adigrat, Ethiopia, with the objective of assessing the prevalence of bovine mastitis, the risk factors associated with the disease and identifying the bacteria responsible for the disease. A total of 322 cows were selected from 10 small holder dairy farms using simple random sampling method. California Mastitis Test (CMT), clinical examination of udder and teats and bacteriological examination were employed. The overall prevalence of mastitis at a cow level was 64.3% (207/322), from which 15 (31/322) and 85% (176/322) were clinical and subclinical, respectively. The quarter level prevalence of the disease was also 54% (696/1288) from which 20.5 (264/1288) and 33.5% (432/1288) were clinical and subclinical form, respectively. As compared to the others, the right hind quarters were affected with the highest infection rate (63.9%). The left hind quarters were the second with an infection rate of 59.3% followed by right front quarters (52.5%) and left front quarters (40.4%). Among the bacterial causes of bovine mastitis in the study area, *Staphylococcus aureus*, *Echerichia coli* and *Streptococcus agalactiae* were the major isolates with percentages of 51.7, 20.9 and 20.3, respectively. All the potential risk factors considered in this study namely, parity, age, stage of lactation and breed showed significant effects on prevalence of mastitis in the present study. The present study concludes that mastitis was a major health problem of dairy cows in the area. Hence, strategic control measures against the disease and regular surveillance measures are recommended.

**Key words:** Prevalence, bovine mastitis, risk factors, bacteria, Adigrat, Ethiopia.

### INTRODUCTION

Ethiopia has the largest livestock population in Africa. Cows represent the largest population of cattle production of the country (CSA, 2007). Hence, development of the dairy sector in Ethiopia can contribute significantly to poverty alleviation and nutrition in the country (Mohamed et al., 2004). Nevertheless, the quality and quantity of milk in the country deteriorates due to various causes. Mastitis is an inflammation of the mammary gland and commonly associated with intra-mammary bacterial infection. It is considered as the most important disease among diseases of the dairy having zoonotic and economic impact (Omore et al., 1996; Al-Majali et al.,

2008).

Bovine mastitis can be clinical with local (in some cases general) clinical signs and milk abnormalities or sub clinical with production losses and lowered milk quality. Clinical signs vary with the severity of the disease and generally include pain, heat and swelling of the affected quarter or half of the gland and abnormality of the milk either as clots or flakes and wateriness of the liquid phase (Radostits et al., 2000).

Bovine mastitis as a disease, has received little attention in Ethiopia, especially the sub clinical form. When modern dairy farming in the tropics was first adopted,

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mastitis was predicted to be important disease in dairy cattle and one of the most tasks of risk factors for animal health and production problems to allow effective control strategies to be adopted (Hunderra et al., 2005).

The disease has been reported by several authors in different parts of Ethiopia (Lakew et al., 2009; Gebreyohannes et al., 2010; Megersa et al., 2010). However, in some parts of Ethiopia, the disease is insufficiently investigated and information relating to its magnitude, distribution and risk factors is scant. Such information is important to envisage when designing appropriate strategies that would help to reduce its prevalence and effects (Megersa et al., 2010; Mekbib et al., 2010).

Even though the disease is known in Adigrat, documented information regarding the disease is unavailable. Therefore, the study was undertaken with the objective of assessing the prevalence of bovine mastitis, the risk factors associated with the disease and identifying the bacteria responsible for the disease.

## MATERIALS AND METHODS

### Study area

The study was conducted in dairy farms in Adigrat town located 898 km away from Addis Ababa in the north east direction, 14° 16' N and 39° 29' E, at an altitude with a range of 246-297 m above sea level. The minimum and maximum temperatures are 9.28°C and 21.91°C, respectively. The area receives a bimodal rainfall of 400 mm minimum and 600 mm maximum (CSA, 2010).

### Study population

Cows from 10 small holder dairy farms of Adigrat were selected using simple random sampling method. The sample size was determined by the formula given by Thrusfield (2005) by assuming the expected prevalence to be 30% (Ministry of Agriculture, Personal information) while the statistical confidence level was 95%. Accordingly, the sample size of lactating cows was determined to be 322 (193 cross bred and 129 local breeds).

### California mastitis test (CMT)

The California mastitis test (CMT) was carried out as a screening test for sub clinical mastitis. It was carried out as per the procedure of Quinn et al. (2004). A squirt of milk, about 2 ml from each half was placed in each of 2 shallow cups in the CMT paddle. An equal amount of the commercial CMT reagent was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane for 15 s. Then depending on CMT results, cases were categorized as either positive based on degree of jell formation or negative which did not show jell formation. Positive CMT-cows were defined as having at least one CMT-positive quarter.

### Sample collection and handling

Samples were collected aseptically as described by Quinn et al. (2004). They were collected before milking. Udders and especially teats were cleaned and dried before sample collection. Each teat

end was swabbed with cotton soaked in 70% ethyl alcohol. Samples were taken in sterile glass vials and closed with screw caps. The vials were marked with a permanent marker, so that the markings were easy to read when the vials were placed in rack. The first streams of milk were discarded and 10 ml of milk was collected into horizontally held vial. After collection, the sample was placed in an icebox and transported to the laboratory for analysis.

### Bacterial culture

Milk samples were examined following standard procedure where about one standard loop full (0.01 ml) of each milk samples was streaked on 10% sheep blood agar. Plates were incubated aerobically at 37°C for up to 72 h and checked for any bacterial growth.

Suspected colonies were identified morphologically, microscopically and biochemically according to Quinn et al. (2004) and NMC (2004).

### Data analysis

The data was entered to Microsoft excel spread sheet and analyzed using statistical software (SPSS version 17) program and total prevalence was calculated by dividing the number of positive cows/quarters by the total number of cows/quarters tested (Thrusfield, 1995). The Chi-square ( $\chi^2$ ) test was applied to test the existence of association between mastitis and risk factors such as breed, age, lactation stage and parity. In all chi-square test applications, a probability level of  $p < 0.05$  was considered statistically significant.

## RESULTS

### Prevalence of mastitis

A total of 322 lactating cows were examined for presence of mastitis out of which 207 (64.3%) cows were found to be affected with clinical and subclinical mastitis based on clinical and CMT diagnosis. Out of the total 207 cows affected, 176 (85%) were positive for subclinical mastitis and 31 (15%) for clinical mastitis.

The result shows that 31(15%) of the cows and 264 (20.5%) of the quarters were found to have clinical mastitis whereas 176 (85%) of the cows and 432 (33.5%) of the quarters were affected with subclinical mastitis. Out of the 1288 quarters examined, 696 (54%) were positive for mastitis (Table 1).

As compared to the others, the right hind quarters were affected with the highest infection rate (63.9%). The left hind quarters were the second with an infection rate of 59.3% followed by right front quarters (52.5%) and left front quarters (40.4%) (Table 2).

### Bacteriological examination

Out of the total samples collected from affected quarters for bacterial isolation, 681 samples showed growth on 10% sheep blood agar. The relative prevalence of various bacterial species isolated from cases is shown in

**Table 1.** Prevalence of clinical and subclinical mastitis at a cow and quarter level.

Form of mastitis	Total examined cows	Total affected cows (%)	Total examined quarters	Total affected quarters (%)
Clinical	322	31 (15%)	1288	264 (20.5%)
Subclinical	322	176 (85%)	1288	432 (33.5%)
Total	322	207 (64.3%)	1288	696 (54%)

**Table 2.** Quarter level prevalence of mastitis.

Quarter	Quarters examined	Affected quarters	Prevalence (%)
Front right	322	169	52.5
Front Left	322	130	40.4
Hind Right	322	206	63.9
Hind Left	322	191	59.3
Total	1288	696	54

**Table 3.** Bacteria isolated from mastitis affected quarters.

Organisms isolated	Frequency	Percentage
<i>Staphylococcus aureus</i>	361	51.7
<i>E. coli</i>	146	20.9
<i>Streptococcus agalactiae</i>	142	20.3
Other gram positive cocci and rods	32	4.6
Other gram negative rods	17	2.5
Total	698	100

Table 3. *S. aureus* was the most predominant isolates constituting 51.7% of all the isolates followed by *E. coli* (20.9%), *S. agalactiae* (20.3%), other gram positive cocci and bacilli (4.6%) and other gram negative rods (2.5%).

### Risk factors for mastitis

Prevalence of mastitis with respect to specific risk factors was determined as the proportion of affected cows out of the total examined. Four variables were considered as potential risk factors for the occurrence of mastitis. The association of mastitis with parity, lactation stage, age and breed was statistically significant ( $p < 0.05$ ). Prevalence of mastitis in cows delivered 1 to 3 calves was 39.7%, four to seven calves was 69.8% and greater than 7 calves was 81.8%.

Mastitis prevalence was highest in early lactation (74.3%) and also higher in late stage of lactation (65%) but lower in mid lactation (46.7%). Cows older than 10 years were affected the highest (79.6%), followed by 6 to 9 years (57.5%) and 1 to 5 years (41.7%). Cross bred cows were affected at higher rate (71%) than local breeds (54.2%) (Table 4).

### DISCUSSION

The present study shows an overall mastitis prevalence of 64.3%, as determined by CMT and clinical examination of udders. The finding is comparable with previous reports of 63% by Geresu (1989) in Addis Ababa and 71% by Mekbib et al. (2010) in Holetta town.

However, the overall prevalence is higher than the previous findings of other authors in different regions of Ethiopia like 34.9% by Biffa et al. (2005) in Southern Ethiopia, 52.8% by Hunderra et al. (2005) around Sebeta and 46.7% by Abera et al. (2010) in Adama. The difference in prevalence reports of mastitis in the present study and other reports could probably be due to difference in breeds, management practices, geographic areas, level of production and differences in study methods.

The prevalence of subclinical mastitis was higher (176, 85%) than clinical mastitis 31(15%). A similar observation of the dominance of subclinical mastitis was observed by several studies: 10.3 and 33.8% by Delelesse (2010); 4.9 and 30.6% by Moges et al. (2012); 19.6 and 55.1% by Zeryehun et al. (2013) for clinical and subclinical mastitis, respectively. The finding strengthened the claim

**Table 4.** Prevalence of mastitis with respect to different risk factors.

Risk factor		Animals examined	Number of animals affected	Prevalence (%)	$\chi^2$ (P-value)
Parity number	1-3 calves	68	27	39.7	23.94(0.000)
	4-7 calves	232	162	69.8	
	> 7 calves	22	18	81.8	
Lactation Stage	Early(1-4 month)	121	88	74.3	13.77(0.001)
	Mid(5-7 month)	75	35	46.7	
	Late (> 7 month)	126	82	65.0	
Age	Young (1-5 years)	24	10	41.7	22.76 (0.000)
	Young adult (6-9 years)	146	84	57.5	
	Old ( $\geq 10$ years)	142	113	79.6	
Breed	Cross	193	137	71.0	8.7 (0.0032)
	Local	129	70	54.2	

made by Ojo et al. (2009) that subclinical mastitis remains the most economically damaging and zoonotic potential disease for dairy industry.

The overall quarter prevalence of mastitis (54%) found in this study was higher than the finding of Mekbib et al. (2010) who reported quarter prevalence rate of 44.9% but lower than the report made by Kifle and Tolossa (2008) in Selale, Ethiopia, Zeryehun et al. (2013) in and around Addis Ababa, Ethiopia, who reported 63.1 and 62.3%, respectively.

As compared to the others, the right hind quarters were affected with the highest infection rate (63.9%). The left hind quarters were the second with an infection rate of 59.3% followed by right front quarters (52.5%) and left front quarters (40.4%). The result shows that the hind quarters are affected more than the front quarters. This could be attributed to the high production capacity of the hind quarters (Radostits et al., 1994) and the high chance of getting fecal and environmental contamination (Sori et al., 2005).

In the study, the predominant organisms isolated from mastitis were *S. aureus* (51.3%) followed by *E. coli* (20.9%) and *S. agalactiae* (20.3%). This is in agreement with the report of Ayano et al. (2013). The predominance and primary role of *S. aureus* isolate in bovine mastitis has also been reported in other studies (Mekbib et al., 2010; Atyabi et al., 2006). The high prevalence of this organism may be associated with its frequent colonization of teats, its ability to exist intra-cellular and localize within micro abscesses in the udder and hence resistant to antibiotic treatment (MacDonald, 1997). The organism is well adapted to survive in the udder and usually establishes a mild sub clinical infection of long duration from which it shed in milk facilitating trans-mission to healthy animals mainly during milking (Radostits et al., 2007).

The study reveals the prevalence of mastitis to be affected significantly ( $p < 0.05$ ) with age. The prevalence of mastitis was highest in old cows (79.6%) followed by young adults (57.5%) and young cows (41.7%). This is in agreement to the findings of previous works by Moges et al. (2011) and Zeryehun et al. (2013). The highest prevalence in older cows is because of their largest teats and more relaxed sphincter muscles, which increase the accessibility of infectious agent in the cows' udder (Radostits et al., 2007). Cows with more than 7 calves (81.8%) were at greater risk than those of cows having 4-7 calves (69.8%) and cows having 1-3 calves (39.7%). Similar finding was reported by Radostits et al. (2007) and Mekbib et al. (2010). Early lactation stage had higher relative prevalence (74.3%) than late (65%) and mid (46.7%) lactation stage; the difference is statistically significant ( $p < 0.05$ ). This result aligns with reports made elsewhere (Delelesse, 2010; Moges et al., 2011; Zeryehun et al., 2013). Absence of dry cow therapy regime could possibly be the major factor contributing to high prevalence at early lactation (Schalm et al., 1971). Cross bred cows were affected (71%) at higher rate than local breeds (54.2%). The difference is statistically significant ( $P < 0.05$ ). This agrees to the findings of Moges et al. (2011).

The present study shows that mastitis was a major health problem of dairy cows in the area hence warrants serious attention. The high prevalence clearly indicated lack of strategic control measures against the disease, as well as poor surveillance measures. It is therefore important that farmers should ensure strict personal hygiene and that of animals, and general sanitary condition of the farms should be improved and maintained. Since the bacteria isolated from cows' milk samples in the present study are types that cause both contagious and environmental mastitis, correct and good milking tech-

niques are essential in the prevention strategies. Furthermore, regular screening for the detection of subclinical mastitis and proper treatment of the clinical cases as well as appropriate treatment of cows during dry and lactation period should be practiced.

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