

## A Cross Sectional Study on the Prevalence of Sub Clinical Mastitis and Associated Risk Factors in and Around Gondar, Northern Ethiopia

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**Abstract:** A cross sectional study was conducted on 322 local (n = 57) and crossbred (n = 265) lactating hand milked small holder cows in and around Gondar. The objective of the study was to determine prevalence of clinical and sub clinical mastitis and the associated risk factors by clinical examination and using california mastitis test. Bacterial culture was also conducted to determine the casual agents of mastitis. The prevalence of clinical and sub clinical mastitis in the study area was 0.93 and 31.67%, respectively with an overall prevalence of 32.6%. Out of 1288 quarters examined 164 were infected while 39 were blind. The number of clinically and sub clinically infected quarters were 7 and 157, respectively. Several pyogenic bacteria have been isolated from cases of sub clinical mastitis during the study period. The major bacteria isolated in this study were *Staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus dysgalactiae* with a prevalence of 16.5, 15.9 and 14.0%, respectively. Among the risk factors considered, breed, age, parity and stage of lactation have been shown statistically significant (p<0.05) difference in the prevalence of mastitis. Sub clinical mastitis in both breeds has been reported to be higher than clinical mastitis. The odds of occurrence of mastitis were two times more likely in crossbreds compared to local zebu. The present study disclosed that high prevalence of sub clinical mastitis and the occurrence *Staphylococcus* and *Streptococcus* spp. as dominant bacterial species from mastitic milk of the study area. All the risk factors considered were significant effect on the prevalence of mastitis. Appropriate control and prevention methods should be applied to reduce the prevalence and effect of mastitis in dairy cows.

**Key words:** Bacterial isolate, clinical mastitis, prevalence, risk factors, sub clinical mastitis

### INTRODUCTION

Ethiopia has a largest livestock population of any African country. Cows represent the largest population of cattle production of the country (CSA, 2007). However, compared to other countries in Africa, Ethiopians consume less dairy products. Moreover, the quality and quantity of milk in the country deteriorates because of various causes. Mastitis is an inflammation of the mammary gland and commonly associated with intramammary bacterial infection. It is considered as one of the most important disease among diseases of the dairy animals (Radostits *et al.*, 2000). Mastitis can be defined as clinical (grossly evident changes to milk, the gland or the whole animal) or as subclinical (diagnosed using ancillary tests such as the somatic cell count). Mastitis is a multifactorial disease, requiring exposure to a combination of environmental and pathogenic factors and with variable responses between animals. Many risk

factors have been identified for clinical and subclinical mastitis in dairy animals such as breed, age, parity, stage of lactation (Fox *et al.*, 1995; Barnouin, and Chassagne, 2001; Compton *et al.*, 2007b).

The disease has been reported by several authors in different parts of the country (Biru, 1989; Bishi, 1998; Nesru, 1999; Mungube, 2001; Lakew *et al.*, 2009; Gebreyohannes *et al.*, 2010; Megersa *et al.*, 2010). Several of these studies have been shown the occurrence a range of mastitis causing bacteria indicating *Staphylococcus* and *Streptococcus* as dominant and pathogenic species. Some authors (Mungube *et al.*, 2005) reported a substantial economic loss in Ethiopian highland crossbred dairy cows due to subclinical mastitis.

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. This paper describes the results of an investigation made to elucidate the prevalence and risk factors of mastitis in lactating dairy cows in and around Gondar.

## MATERIALS AND METHODS

**Study area:** The study was conducted on randomly selected cows between September 2010 and February 2011 in and around Gondar town, North Gondar Administrative Zone of Amhara National Regional State (ANRS). Gondar is located in the north-western part of Ethiopia, 710 km North West of Addis Ababa. The study area is found at 12°40' N Longitude and 37°45' E, Latitude with an altitude range of 1802-2200 m above sea level. The soil type falls into three categories: Heavy black clay soil, loam brown and red soil and sandy loam soil. The ranges of maximum and minimum temperature vary between 22-30.7°C and 12.3-17.1°C, respectively. The region receives a bimodal rainfall, the average annual precipitation rate being 1000 mm. The short rains occur during the months March, April and May while the long rains extend from June through September (MOA, 2004).

**Study animals and husbandry practices:** The study population comprises of approximately 322 crossbred ( $n = 265$ ) and lactating Zebu cows ( $n = 57$ ) in and around Gondar town managed under semi-intensive and extensive production system. In the semi-intensive production system, the animals are mainly composed of crossbred cattle. They are kept indoors and graze in the field occasionally. They are supplemented with concentrates in addition to hay. On the other hand, the extensive production system that consists of local breeds that depend for feed on grazing at the field with minor supplementation in the evening when they come back home. Local dairy cows are managed under traditional and extensive husbandry systems. The animals are relatively smaller in size and have small udder and short teats. The average daily milk production from individual cows was relatively low (4 to 5 L). Crossbred dairy cows are often managed under a small-scale, semi-intensive management system. They are often provided with some supplementary diet in addition to the natural pasture and crop by products and are maintained usually in separate stalls a short distance from each other in a house. This type of dairy husbandry system is increasingly becoming an important source of milk supplies to households and a means of income generation in urban and peri-urban areas of Gondar. Manure removal is generally made on a daily basis. Although milking is done by hand, pre-milking and post-milking hygienic procedures, such as udder washing and drying are not practiced. Cows are dried off at late-lactation abruptly.

### Study design:

**Prevalence study:** The study was a cross sectional study carried out in and around Gondar town. Grades of the CMT were evaluated and the results graded as 0 and 1 for negative and 2 and 3 for positive. Disease identification was made based on clinical examination, nature and appearances of milk secretion, and reaction to CMT. Accordingly, milk with pus flakes, clots, or blood-tinged watery secretion, yet no visible or palpable changes in mammary quarters, and acute mastitis with signs of systemic involvement were diagnosed as clinical mastitis. Sub clinical mastitis was diagnosed based on CMT results and the nature of coagulation and viscosity of the mixture, which show the presence and severity of the infection, respectively. This study also incorporated prevalence of blind/blocked quarters.

**Sample size and sampling method:** Sample size was determined at 95% confidence interval, 5% precision and from previous studies in similar study area (Workineh *et al.*, 2002), with an expected prevalence of 30%. The sample size was determined by using the formula for simple random sampling (Thrusfield, 1995).

The sample size thus determined was 322 animals to be drawn from the study population. However, because of the difficulties of recording all lactating cows, sampling frame, and considering the number of cows in each household on the average to be 2 cows, the number of households selected was determined by dividing sample size with herd size, in this case  $322/2 = 161$ . Hence, these study 161 households were included.

**Data collection:** A questionnaire was developed, pre tested and administered to the owners of the animals. Data on each cow was collected in a format designed for this purpose. Relevant information was collected on cow history, housing system, milking practice, drug usage and other management practices. Risk factors considered were breed, age, parity, stage of lactation, tick load or lesion and husbandry system. Depending on clinical inspection and CMT results, cases were categorized as either positive or negative. Positive cases were further categorized as clinical and sub clinical mastitis.

**Sampling equipment:** 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 a rack. The vials were marked before sampling. The surface of the teat ends were sterilized by wiping with clean cotton dipped in 70% alcohol. An insulated cool box was used for transporting samples.

**Clinical inspection of the udder:** The clinical inspection of the udder was done in the following way. The udder was first examined visually and then by palpation to detect fibrosis, inflammatory swellings, visible injury, tick infestation, atrophy of the tissue, and swelling of supra mammary lymph nodes. The size and consistency of mammary quarters were inspected for the presence of any abnormalities, such as disproportional symmetry, swelling, firmness, and blindness. Mammary quarters often became blind when exposed to repeated infections and little or no treatment was provided. Information relating to the previous health history of the mammary quarters and causes of blindness was obtained from interviews with owners of the farm. Viscosity and appearance of milk secretion from each quarter were examined for the presence of clots, flakes, blood, and watery secretions. The udder was also inspected for the presence of any grossly visible injury and ticks. Injuries caused by ticks and vigorous calf suckling were described based on location, size, and nature. Injuries caused by ticks were identified as indurate necrotic lesions following detachment of the ecto parasites; these could be with or without abscess formation. Injuries caused by vigorous calf suckling were identified as circumscribed lesions around the teats.

**Preparing udders and teats:** Udders and especially teats were cleaned and dried before sample collection. Each teat end was scrubbed vigorously with cotton or gauze sponge moistened (but not completely wet) with 70% ethyl alcohol. Recontamination of teats during scrubbing was avoided, the teats on the far side of the udder first, then those on the near side. A separate pledged or sponge was used for each teat. Scrubbing was continued until a new surface of the cotton or sponge remains clean.

**Collection of milk samples:** Procedures for collecting milk sample were according to (Schalm *et al.*, 1971; Sears *et al.*, 1993; Quinn *et al.*, 1994). Strict aseptic procedures were used when collecting milk samples in order to prevent contamination with the many microorganisms present on the skin of cow's flanks, udder and teats, on the hands of the sampler, and in the barn environment. Teats towards sample collection were taken first and then the far ones. The first 3-4 streams of milk were discarded. The collecting vial was as near horizontal as possible and by turning the teat to a near horizontal position, 15 mL of milk was collected into the vial.

**Time of sample collection:** Samples for culture were collected before milking that was most convenient under the management conditions of the individual herd.

**Handling and storing samples:** After collection, samples for cultures were placed in racks for ease of handling and held in an icebox, properly packed and kept cold. They were processed as soon as possible.

**California Mastitis Test (CMT):** The California mastitis test was carried out as screening test for selections of samples for culture following the method described by Schalm *et al.* (1971) and Quinn *et al.* (1994). A squirt of milk, about 2 ml from each quarter was placed in each of four shallow cups in the CMT paddle. An equal amount of the commercial reagent was added to each cup. A gentle circular motion was applied to the mixtures, in a horizontal plane for 15 sec. The reaction was interpreted according to Schalm *et al.* (1971) and Quinn *et al.* (1994).

**Data analysis:** All data collected were entered into Microsoft excel spreadsheet. The prevalence of sub-clinical mastitis was the dependent variable while parity, stage of lactation, herd size and presence of teat lesion were independent variables considered at cow level. The independent variables at herd level included barn floor status, milking hygiene and milking strategy. The associations between dependent and independent variables were tested by logistic regression.

For analysis of the data SPSS version 15 and EPI-INFO 2006 software's were used. In this chi-square test, odds ratio, confidence intervals and logistic regression (linear model) were calculated.

## RESULTS

**Prevalence:** A total of 322 lactating cows (57 indigenous zebu and 265 Holstein × zebu) crosses in small holder dairy farms in Gondar milk shed were investigated to determine the magnitude of mastitis. Out of 1288 quarters examined 39 (3.02%) were blocked. Clinical prevalence at cow level was 3 (0.93%) in cross breeds and none in local zebu breeds (Table 1).

The prevalence of sub clinical mastitis was determined by CMT and by microbiological culture (Table 2). Sub clinical mastitis at cow level was 34.72% in cross breeds and 17.54% in indigenous zebu using CMT (Table 2). Based on culture sub clinical mastitis prevalence at cow level was 30.57% in cross breeds and 15.78% in indigenous zebu (Table 3).

Based on CMT the prevalence of quarter level sub clinical mastitis in cross breed and local zebu cows was 16.05% and 5.12% respectively. Based on culture the prevalence of quarter level sub clinical mastitis in cross breed and local zebu cows was 14.99 and 4.19%, respectively (Table 4 and 5).

Table 1: Prevalence of clinical mastitis at cow level in crossbred and local zebu lactating cows

Breed	No. of animals examined	Status of clinical mastitis		Prevalence % & 95% CI
		Positive	Negative	
Cross	265	3	262	1.13 (0.0, 2.4)
Local zebu	57	0	57	0
Total	322	3	319	0.93 (0.0, 1.9)

Table 2: Prevalence of sub clinical mastitis at cow level using CMT in crossbred and local zebu lactating cows

	No. of animals examined	Status of sub clinical mastitis		Prevalence % & 95% CI
		Positive	Negative	
Cross	265	92	173	34.72 (28.98, 40.45)
Local zebu	57	10	47	17.54 (7.67, 27.42)
Total	322	102	220	31.68 (26.59, 36.78)

Table 3: Prevalence of sub clinical mastitis at cow level using culture in crossbred and local zebu lactating cows

Breed	No. of animals sampled	Status of sub clinical mastitis		Prevalence % & 95% CI
		Positive	Negative	
Cross	265	81	184	30.57 (25.02, 36.11)
Local zebu	57	9	48	15.78 (6.32, 25.26)
Total	322	90	232	27.95 (23.05, 32.86)

Table 4: Prevalence of sub clinical mastitis at quarter level using CMT in crossbred and local zebu lactating cows

Breed	No. of animals sampled	Status of sub clinical mastitis		Prevalence % & 95% CI
		Positive	Negative	
ross	1034	166	868	16.05 (13.82, 18.29)
Local zebu	215	11	204	5.12 (2.17, 8.06)
Total	1249	176	1073	14.09 (12.16, 16.03)

Table 5: Prevalence of sub clinical mastitis at quarter level using culture in crossbred and local zebu lactating cows

Breed	No. of animals sampled	Status of sub clinical mastitis		Prevalence % & 95% CI
		Positive	Negative	
Cross	1034	155	879	14.99 (12.81, 17.17)
Local zebu	215	9	206	4.19 (1.51, 6.86)
Total	249	164	1085	13.13 (11.26, 15.00)

Table 6: Risk factors for the occurrence of sub clinical mastitis odds ratio and p-values as estimated by the logistic regression equation

Variable	Odds ratio	p-value
Breed	2.331	0.000
Age	1.344	0.005
Parity number	1.566	0.009
Stage of lactation	1.710	0.000
Tick /or lesion	-	0.065
Husbandry system	-	0.715

Both at cow and quarter level, prevalence were high in cross breeds compared to locals. The difference was statistically significant ( $p < 0.05$ ).

**Risk factors affecting prevalence of mastitis:**

Prevalence of mastitis related to specific risk factors was determined as the proportion of affected cows out of the total examined. Six variables were considered as potential risk factors for the occurrence of sub clinical mastitis in this study. These risk factors were (breed, age, parity,

lactation stage, and tick load or lesion and husbandry practice). The association of mastitis with the risk factors was investigated using chi-square ( $\chi^2$ ) and odds ratio (OR).

Breed, age, parity and stage of lactation were statistically significant ( $p < 0.05$ ). Crosses of zebu with Holstein Frisian cows were affected (71.8%) at higher rate than zebu breeds (28.2%); the difference is statistically significant ( $p < 0.05$ ). Older cows greater than 8 years were more affected sub clinical mastitis (79.6%) than younger cows (20.4%); the difference is statistically significant ( $p < 0.05$ ). Prevalence of sub clinical mastitis in cows delivered 1 to 3 calves was (14.2%), 4 to 6 calves (31.6%) and greater than 6 calves (54.2%); the difference is statistically significant ( $p < 0.05$ ). Mastitis prevalence was highest in early lactation (47.3%) and also higher in late stage of lactation (39.9%) but low in mid lactation (12.8%); the difference is statistically significant ( $p < 0.05$ ) (Table 6).

## CONCLUSION

Prevalence of bovine mastitis in smallholder farms in and around Gondar was too high. This shows that bovine mastitis is an important dairy health problem in this area. Sub clinical mastitis in both breeds was found higher than clinical mastitis. Breed, age, parity, and stage of lactation were the risk factors. *Staphylococcus aureus* and *Coagulase negative staphylococci* were the major mastitis inducing pathogens detected.

Usually Ethiopian dairy holders especially smallholder are not well informed about the invisible loss from sub clinical mastitis, this form of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases while the high economic could come from sub clinical mastitis.

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