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Prevalence of clinical and sub-clinical mastitis on cross bred dairy cows at Holleta Agricultural Research Center, Central Ethiopia

Ararsa Duguma[1*], Tadele Tolosa[1] and Aster Yohannes[2]

[1]Jimma University, College of Agriculture and Veterinary Medicine,P.O.BOX, 307, Jimma, Ethiopia.
[2]Holleta Agricultural research center, Central Ethiopia.

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A study on bovine mastitis, designed to determine the prevalence and causal agent in cross breed dairy cow, was conducted from November, 2009 to March, 2010 at Holleta agricultural research center. A total of 90 cross bred cows were examined by physical examination and California mastitis test (CMT). Out of the total animals examined, 81.1% (73) had mastitis, in which 7.8% (7) and 73.3% (66) had clinical and sub clinical mastitis, respectively. Out of 340 quarters examined, 80.88% (275) were found to be infected in which 5.59% (19) were clinically and 75.3% (256) were sub clinically. Of 275 CMT and physically positive animals, quarter samples were taken for microbiological test in which, 66.4% (180) were culturally positive and 33.09% (91) were negative. Of 180 positive samples, the majority of isolates were *Staphylococcus aureus* (43.3%), followed by *Micrococcus* spp. (17.2%), *Streptococcus agalactiae* (12.2%), and *Streptococcus dysgalactiae* (7.2%). In addition, lowest isolation rate was for *Streptococcus uberis* and *Streptococcus feacalis* and each accounts for 2.8%. From this, proper mastitis control should be practiced by maintenance of an appropriate cow's environment and udder health management program in the farm including further investigation on risk factors associated to prevalence of mastitis and antibiotic resistance test to undertake measurable control options of mastitis in the farm.

Key words: Cross bred, dairy cow, causal agent, holleta, mastitis, prevalence.

INTRODUCTION

In Ethiopia, livestock represents a major national resource and form an integral part of the agricultural production system. The country has the largest livestock population of any African country with estimated 43.1 million heads of cattle and cows representing the largest proportion of indigenous cattle of the country (Central Statistical Authority (CSA), 2008).

Milk produced from these animals provides an important dietary source for the majority of rural as well as a considerable number of the urban and peri-urban population. However, milk production often does not satisfy the country's requirements due to a multitude of factors, out of which disease of the mammary glands known as mastitis is among the various factors contributing to reduced milk production (Fekadu, 1995). Mastitis can be defined as clinical (grossly evident changes

*Corresponding author. E-mail: ararsad@yahoo.com

to milk, the gland or the whole animal) or as subclinical (diagnosed using ancillary tests such as the somatic cell count).

Bovine mastitis can be caused by physical or chemical agents but the majority of cases are infectious and usually caused by bacteria. The disease has been reported by several authors on the prevalence and major causes of bovine mastitis mostly in cross bred dairy cattle in different parts of the country (Workineh et al., 2002; Biffa et al., 2005; Sori et al., 2005; Mungube, 2001). Several of these studies have shown the occurrence as a range of mastitis causing bacteria, indicating *Staphylococcus* and *Streptococcus* as dominant and pathogenic species.

Despite many years of research, mastitis remains the most economically damaging disease for dairy industry worldwide (Owens et al., 1997). Most estimates show that on the average an affected quarter suffers a 30% reduction in productivity and an affected cow loss of 15% of its production for the lactation.

In Ethiopia, the disease has been studied sufficiently, and information relating to its prevalence and risk factors are limited to some areas with a variable results. For this reason, more and exact knowledge from expanded epidemiological analysis of mastitis is needed for creating better control program. Efforts have only been concentrated on the treatment of clinical case.

The disease is worth studying as it causes financial losses attributed to reduced milk yield, discarded milk following antibiotic therapy, early culling of cows, veterinary costs, drug costs, increased labor, death in per acute septicemia, and replacement costs (Nesru et al., 1997).

Regular and systematic studies of Mastitis should be carried out in order to make information on the prevalence of the disease available and put forward an appropriate disease control strategies for this economically important disease. Therefore, the present study was undertaken to determine the prevalence of mastitis and isolation of predominant bacteria causing clinical and subclinical mastitis in cross bred lactating dairy cows of Holleta agricultural research center.

MATERIALS AND METHODS

Study area

The study was conducted at Holleta agricultural Research in the central high lands of Ethiopia. Holleta is located in central highland of Oromia special zone surrounding Finfine at a latitude of 38° 30'E, 9° 3'N and 29 km west of Addis Ababa on high way to Ambo. It has an altitude of 2400 m above sea level and receives mean annual rain fall of 1100 mm with bimodal distribution 70% of which occurs during the main rainy season (June to September) and 30% during the small rainy season (February to April) and the annual temperature of 11 to 22°C with relative humidity of 50.4%.

Study animals and husbandry practice

The study animals include crossbred dairy cows (Borana × Holstein breed) owned by Holleta Agricultural research center. The animals were often managed under a semi-intensive management system. They are often provided with some supplementary diet in addition to the natural pasture and agricultural byproducts and some are maintained usually in separate stalls, a short distance from each other in a house. Although milking was done by machine and hands; pre-milking and post-milking hygienic procedures, such as udder washing and drying, were frequently practiced. Cows were allowed to dry off at late-lactation period by abrupt cessation of milking.

Study design and sampling technique

Cross-sectional study was conducted from November, 2009 to March, 2010 in lactating dairy cows to determine the prevalence and identification of the pre dominant bacteria causing clinical and sub clinical mastitis in the study area. All lactating crossbred dairy cows were examined using clinical inspection and CMT results. From positive animals, milk samples were collected for bacterial culture and isolation.

Sample size

All lactating dairy cows of the farm (90 in numbers) represented by 360 samples at quarter level were examined and from positive animals milk samples were collected for bacterial isolation at quarter level.

Data collection

Data such as abnormal changes in the milk, mammary gland and CMT score were collected during animal examination. Depending on this clinical inspection and CMT results, cases were categorized as either positive or negative and positive case was further categorized as clinical and sub-clinical mastitis.

Detection of mastitis

Mastitis was detected using the California mastitis test (CMT) and results of clinical inspection of udder based on 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 of (E.E.C. scientific limited Northampton, U.K. product) was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane for 15 s. Accordingly, milk with pus flakes, clots or blood-tinged watery secretion, and acute mastitis with signs of systemic involvement was 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.

Preparing udders and teats

The udders, especially teats were cleaned and dried before sample collection. Each teat end was scrubbed vigorously with a pledged of cotton moistened (but not completely wet) with 70% of ethyl alcohol. Recontamination of teats during scrubbing was avoided by

Table 1. Prevalence of clinical and sub clinical mastitis at cow and quarter levels**.**

Form of mastitis	At cow level		At quarter level	
	No. examined	Affected no. (%)	No. examined	Affected no. (%)
Clinical	90	7(7.8)	340	19(5.59)
Sub clinical	90	66(73.3)	340	256(75.29)
Total	90	73(81.1)	340	275(80.88)

Table 2. Quarter prevalence of clinical and sub clinical mastitis.

Quarter	Clinical mastitis		Sub clinical mastitis	
	No. examined	Positive and prevalence (%)	No. examined	Positive and prevalence (%)
RF	83	6(7.2)	83	64(71.1)
RH	87	5(5.7)	87	65(74.7)
LF	84	3(3.6)	84	63(75.0)
LH	86	5(5.8)	86	64(74.4)
Total	340	19(5.6)	340	256(75.3)

*****RF= right front, RH = right hind, LF = left front, LH = left hind.

scrubbing, and separate pledged cotton was used for each teat.

Milk sample collection

During milk sample collection, teats towards sample collection were sampled first and then the far ones. The first 3 to 4 streams of milk were discarded. The collecting vial was held as near horizontal as possible, and by turning the teat to a near horizontal position, approximately 10 ml of milk was collected into a universal sample collection bottle. After collection, the samples were placed in icebox and taken to Holleta agricultural research center microbiology laboratory for bacterial culture and isolation within 7 to 10 days.

Bacterial culture and isolation

From the samples, a standard of 0.01 ml of milk was streaked on blood agar plate (Himedia Laboratories Pvt. Ltd) and incubated aerobically at 37°C and observed within 24 to 72 h for growth of bacteria causing mastitis. Identification of the bacteria on blood agar plate was done based on colony morphology, haemolysis (type of haemolysis, presence or absence of haemolysis), staining technique, coagulase test and catalase test. The colonies was sub-cultured on selective media such as Edward's media (India product) to identify *Streptococcus* bacteria, manitol salt agar base (product of DIFCO Laboratories Detroit 1 Michigan USA) for *Staphylococcus*. Other biochemical tests used to identify the predominant cause of bovine mastitis were used based on that of Quinn et al. (1994).

Data management and analysis

All collected data were entered in Microsoft excel sheet and analyzed by dividing positive cases for a total examined to determine the prevalence of mastitis and bacterial isolates in the study farm.

RESULTS

Prevalence of mastitis by clinical examination and CMT test

A total of 90 animals including only cross bred lactating cows were examined; out of which 73 (81.1%) cows were found to be affected either with clinical or sub clinical mastitis based on the clinical diagnosis and CMT. Likewise, CMT positive for the sub clinical mastitis were found to be 66 (73.3%) (Table 1). Out of the 360 quarters examined, 20 (5.56%) quarters were blind teat. Upon screening of the functional teats (340), a quarter of 275 (80.88%) were affected by clinical and Sub-clinical mastitis. The quarter prevalence of clinical and sub clinical mastitis of all teats were relatively affected equally and the overall quarter prevalence was 75.3% for sub clinical and 5.6% for clinical ones (Table 2).

Bacterial isolates

Milk sample of 271 at quarter level from positive cases were collected and cultured for microbiological examination and 180 (66.4%) were found or yielded bacteria in which 6 (2.2%) of them showed mixed growth; whereas 91 (33.58%) did not grow. From the positive samples, 11 (4.05%) were from clinical and 169 (62.4%) were from sub clinical cases (Table 3). The predominant isolated bacteria were *Staphylococcus* spp. with isolation rate of 47.2% and followed by *Streptococcus* spp., with isolation rate of 25.0%. *Micrococcus* spp. were the third

Table 3. Bacterial species isolated from Bovine clinical and sub clinical mastitis.

Bacterial isolates	Clinical mastitis	Sub clinical mastitis	Total
	No. (%)	No. (%)	No. (%)
Staphylococcus aureus	5(45.5)	73(43.2)	78(43.3)
CNS	0(0)	7(4.1)	7(3.9)
Micrococcus sp	0(0)	31(18.3)	31(17.2)
Streptococcus agalactiae	3(27.3)	19(11.2)	22(12.2)
Streptococcus dysglactiae	0(0)	13(7.7)	13(7.2)
Streptococcus uberis	0(0)	5(2.96)	5(2.8)
Streptococcus faecalis	2(18.2)	3(1.8)	5(2.8)
Actinomyces pyogenes	1(9.0)	6(3.6)	7(3.9)
Corynebactrium bovis	0(0)	6(3.6)	6(3.3)
Mixed growth	0(0)	6(3.6)	6(3.3)
Total	11(100)	169 (100)	180(100)

predominance isolated with isolation rate of 17.2%.

DISCUSSION

The current study showed high prevalence of mastitis 81.1%, which is relatively comparable with Mekibib et al. (2010) at Holleta area who indicated the cow level overall prevalence of 71.0%. However, it is higher than the reports of Lemma et al. (2001) who indicated that 64.5% were positive for mastitis at cows' level, 40% in South Ethiopia by Kerro and Tareke (2003), 44.1% by Girma (2010), The variability in the prevalence of bovine mastitis between reports could be attributed to differences in management of the farms, breeds considered, or technical know-how of the investigators (Radostits et al., 2007). The higher prevalence of bovine mastitis in the present study farm may be due to management practices and infectious agents, having different causes, degrees of intensity, and variations in duration and residual effects.

In this study, the clinical mastitis accounted for 7.8% whereas the sub-clinical mastitis was 73.3% of the share. The result agreed with 7.14% which was reported by Tsegai (1997), whereas the prevalence rate for clinical mastitis obtained in this study area is higher than the finding of Enyew (2004) (3.9%) from Bahir Dar, Ethiopia.

In case of sub clinical mastitis, the prevalence rate (73.3%) obtained in this study was comparable with the finding reported by Zerihun (1996) who reported 68.1%. Sub-clinical mastitis has been reported to be higher than clinical mastitis owing to the defense mechanism of the udder, which reduces the severity of the disease (Radostits et al., 2007). Another is little attention given to subclinical mastitis while treating clinical cases. Moreover, farmers in Ethiopia are not well informed about the silent cases of mastitis (Karimuribo et al., 2006). The quarter prevalence of 75.3% for sub clinical mastitis obtained by this study indicates the economic significance of the disease. The prevalent found in front and hind quarters showed no great difference which agrees to the finding of Gezahegne (1999). However, slight higher prevalence of hind quarters in the present study is due to general production quality of hind quarters.

The result obtained from bacteriological analysis of the sample revealed that the predominant organisms isolated from clinical and sub clinical mastitis were staphylococci, accounting for 47.2%. From a total of 180 isolates, *Staphylococcus aureus* was the most frequently encountered organism with an isolation rate of 43.3%. The predominance and primary role of *S. aureus* isolate in bovine mastitis has also been reported in other studies (Atyaib et al., 2006; Fadlelmoula et al., 2007; Mekbib et al., 2010). The reason for higher isolation rate of *S. aureus* is the wide ecological distribution inside the mammary gland and skin. In areas where hand milking and improper use of drug is practiced to treat mastitis case, its dominance has been suggested. *S. aureus* is adapted to survive in the udder and usually establishes mild sub clinical infection of long duration from which it is shed through milk serving as source of infection for other healthy cows and transmitted during the milking process (Radostits et al., 2007). Hence, the organism has been assuming apposition of major importance as a cause of bovine mastitis.

Streptococcus spp. was the second prevalent bacterial species isolated with isolation rate of 25%. Radostits et al. (1994) stated that *Streptococcus* spp. is the most prevalent along with *Staphylococcus* spp. However, the lower prevalence as compared to *Staphylococcus* spp. is because *Streptococcus agalactiae* survives poorly outside the udder, and established infections are eliminated by frequent use of penicillin and other antibiotics. *Micrococcus* spp. was isolated in the rate of 17.2%,

making it the third most prevalent isolate. *Micrococcus* spp. is considered as a normal teat flora with minor pathogenecity (Radostits et al., 1994) as was observed by Mekbib et al. (2010) in Central Ethiopia.

The isolation of *Corynebacreum* spp. 3.3% was lower than the report of Nejib (2008), who found (6.57). However, the present finding was higher than the finding of Shipigel (1998) and Hamir et al. (1978) who reported 1.3 and 2.5%, respectively. *Actinomyces pyogenes* was isolated at a rate of 3.9% which is comparable with Nejib (2008) who reported 2.92% from his study on survey and isolation of major bacteria causing mastitis in and around Western Harerghe of Ethiopia.

Conclusion

This study showed high prevalence of Bovine mastitis and is a major health problem of dairy cows in the study farm and undoubtedly will have an adverse effect on productivity of dairy industry and hence need serious attention. The major bacterial isolate from positive samples were *Staphylococcus* spp. followed by *Streptococcus* and *Micrococcus* spp. including some environmental pathogens. Based on this, proper mastitis control should be practiced by maintenance of an appropriate cow's environment and udder health management program, and further investigation should be continued with special emphasis on risk factors associated with prevalence of mastitis and antibiotic resistance test to undertake measurable control options of mastitis in the farm.

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