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Full Length Research Paper

Study on prevalence of bovine mastitis in lactating cows and associated risk factors in and around Areka town, Southern of Ethiopia

Lidet G/Michael[1], Benti Deressa[1], Feyissa Begna[1] and Abebe Mekuria[2]*

[1]College of Agriculture and Veterinary Medicine, Jimma University, Ethiopia.

[2]College of Veterinary Medicine, Mekelle University, Ethiopia.

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A cross-sectional study was conducted from October 2011 to March 2012 on a total of 384 lactating (Zebu 183, Jersey103 and Holstein 93) dairy cows randomly selected from 29 rural kebele in which three small scale dairy farms were included. The study was designed with the objective to determine the prevalence of mastitis and isolate the major bacteria that causes mastitis involved in areka woreda and its surrounding wolayta zone. Milk samples was collected and bacteriological culture was done and further confirmation was done by BIOLOG identification system. The overall prevalence of mastitis in the area was 52.9% (n = 203), out of which 9.4% (n = 36) were clinical and 43.5% (n =167) were sub-clinical cases. Among the isolated bacterial genera, the isolate were *Staphylococcus* (14.8%), *Streptococcus* (7.5%), *Corynebacterium* (0.52%) and *coliform* (0.25%). Characterization was also under taken and the species recovered were *Staphylococcus aureus* 136 (54.4%) dominating followed by *Streptococcus dysagalactiae* 62 (24.8%), *Staphylococcus intermidius* (8.4%) *Streptococcus.uberis* 13 (5.2%) *Staphylococcus epidermides* (4.4%) *Streptococcus agalactia* 4 (1.6%), *Corynebacterium pyogens* 2 (0.8%) and *Escherichia coli* 1 (0.4%). There was no statically significant variation (P>0.05) between breeds and the parity number of the cow, but the prevalence of mastitis was found to be statistically significantly among different age groups and lactation stages (p<0.05). The study shows that mastitis is significant problem of dairy cows in the study area and the major isolated bacteria were contagious pathogens. Therefore, hygienic milking practice, culling of chronically infected cows and hygienic practice in the environment should be followed.

Key words: Bovine, mastitis, prevalence, Areka Woreda, SNNPR, Ethiopia.

INTRODUCTION

Mastitis is complex disease that generally involves interplay between management practices and infectious agents, having different degrees of intensity and variations in duration and residual effects. Various infectious agents numbering more than twenty different groups including bacterial, viruses, yeast, fungi and

*Corresponding author. E-mail: abebemekuria28@yahoo.com. Tel: +251 912260385.

rickettsia with bacterial being the major cause have been reported (Biffa et al., 1999). At least, 137 infectious causes of bovine mastitis are known to date and in large animals, the commonest pathogens are *Staphylococcus aureus, Streptococcus agalactiae,* other *Streptococcus* species and coliforms (Sumathi et al., 2008). It may also be associated with many other organisms including *Actinomyces pyogenes*, *Pseudaomonas aeruginosa*, *Nocardia asteroides*, *Clostridium perfringens* and others like *Mycobacterium*,*Mycoplasma*, *Pastuerella* and *Prototheca* species and yeasts (Rodostits et al., 2007).

In Ethiopia, the incidence and distribution of the disease has not been studied systematically and information relating to economic loss and the overall prevalence of the disease is inadequate. The economic losses due to mastitis that the state farms in Ethiopia are experiencing are not difficult to imagine as more than 10% of cows in most farms have at least one blind quarters (Goshu et al., 1985).

The environmental condi-tion of Areka woreda is assumed to be one of the representatives of mid high lands of Ethiopia. During the rainy seasons, the environment assumes muddy wet, humid and moisture conditions which favors the multi-plication and grown of various microorganisms and potentiate their disease producing capacity. Therefore, the aim of this study was to isolate the major causative agent and to assess the major risk factors responsible for mastitis in the study area.

MATERIALS AND METHODS

Study area

The study was conducted in Areka woreda which is in SNNPR in Wolaita Zone located 360 km from Addis Ababa, the capital of Ethiopia. The area is bounded with Damot Gale Woreda to the East, Damot soria Woreda to the south, Balso bomba Woreda to West and Hady Hadero Woreda to the north. Its altitude ranges from 1650 to 2980 (m.a.s.l). It receives an annual rainfall ranging from 1000 - 1200 mm and an annual temperature range of 25-35°C. The area is categorized under Woina-dega agro-ecological climate.

Study design

Cross-sectional study was conducted from October 2011 to April 2012 in Areka woreda.

Study population

The study populations were lactating cows of different age, lactation stage, parity and breed of some dairy farm and smallholder farms of the town including surrounding Keble. Representative Keble was selected using simple random sampling methods.

Sample size

The sample size was determined according to the formula given by Thrusfield (2005) by taking pervious prevalence of mastitis 50%. Accordingly, the calculated value for sample size is equal to 384. Data regarding the different potential risk factors (age, parity, lactation stage, housing conditions and previous history of mastitis) was collected for 384 lactating cows from farm records when available and by interviewing the farm owner when not available.

Milk sample collection

A milk sample was collected according to the National Mastitis Council (NMC) (1990).

Culture and identification

Bacteriological examination was done according to the National Mastitis Council Guideline (1990). Finally, pure colony was taken and sub-cultured on BUG (BiOLOG Universal Growth Media) at 37°C for 18-24 h as a primary and secondary culture. Well-isolated fresh colonies from BUG (Biolog, USA) media are inoculated into 18-20 inoculation fluid to have bacterial suspension with turbidity equivalent to 20% transmittance as measured by turbidity meter. This suspension was poured into of Micro plates with multi-channel pipettes. The Micro Plates were loaded into Omnilog tray to be incubated, analyzed and interpreted for 18-24 h as per guidelines of BiOLOG Users Guideline (2008) and finally identified bacteria was obtained and printed out.

Statistical analysis

The data collected and recorded on specifically designed forms and prepared for analysis was entered in the Microsoft excel spread sheet and analyzed with SPSS version 16 statistical software. Descriptive statistic was used to summarize the data generated from the study. The prevalence of mastitis (clinical and sub-clinical) was calculated by using percentage values and possible associa-tion of disease with risk factors was analyzed by using Chi-square test and predictive value (P-value).

RESULTS

A total of 384 dairy cows were examined for the presence of mastitis both clinically and by the use of screening test, California Mastitis Test (CMT) supported by detailed bacteriological examinations and the results were sum-marized in the following subtitles. The overall prevalence of the disease was 52.9%.

Prevalence of mastitis by breed

Breed difference can play a vital role in the prevalence of different diseases. In this study area, different breeds of cows are there especially at farm level (Table 1). The pre-

Table 1. The prevalence of mastitis by breed.

Breed	Positive no. (%)	Negative no. (%)
Jersey	54(52.4)	49(47.6)
Zebu	91(49.7)	92(50.3)
Holstein	58(55.9)	40(43)
Total	203(52.9)	181(47.1)

$X^2 = 2.297$, P = 0.309.

prevalence of the three breeds namely: indigenous zebu, Holstein and Jerseys was found statistically not significantly different (P>0.0.5).

Prevalence of mastitis based on lactation stage

With regard to the inflammation of the udder of different causes, the lactation stage plays an important role and it was considered as one risk factor for the prevalence of the mastitis in this study. The difference in the presence of mastitis in the study population according to the category set was statistically significant indicating that those animals at the end stage of lactation were affected more (Table 2).

Prevalence of mastitis based on the number of parity

The prevalence measure based on the number of the parity was statistically not significant. The infection generally increases with increasing lactation number. According to this study, the higher occurrence of infection, 58.3% was in 3rd and 4th parity groups and lower in 5th and 6th parity groups (Table 3).

Prevalence of mastitis based on different age groups

Age is a detrimental factor in the distribution of the diseases because at some time it is stressor. It was taken into consideration and the prevalence of mastitis was measured for different age groups of lactating cows. The prevalence was found to be much higher in the young and adult age group than the older age group (Table 4). This is actually found to be statistically significant with a P-value of 0.023.

Isolation of bacteria from clinical and sub clinical mastitis cases

In the present study, mastitis causing bacteria were isolated from clinical and/or sub clinical mastitis cases. Among the bacterial spices, *Staphylococcus Aureus* 136 (54.4%) dominated followed by *Sterptococus dysgalactia* 62(24.8%), *Staphylococcus intermidius* 21 (8.4%), *Sterptococus uberis* 13 (5.2%) *Staphylococcus epidermides* 11(4.4), *Sterptococus agalactia* 4 (1.6%), *Corynebacterium pyogen* 2 (0.8%) and *E. coli* 1 (0.4%) which was isolated from clinical and sub clinical mastitis cows (Table 5).

DISCUSSION

The study was carried out to determine the prevalence of bovine mastitis and to identify the major bacteria that causes mastitis in Areka woreda and it was revealed that 52.8% animals examined had infections in their udders as evidence of mastitis. This finding closely agrees with those of Handera et al. (2005) and Salih et al. (2011) who reported the prevalence of 52.78% in Ethiopia and 52% in Nigeria, respectively. The current study's prevalence was lower than the finding of Abaineh (1997) who reported 65% in fiche and was higher than the finding of Tolosa et al. (2009), Biffa (1994), Shimel (1990), Darsema (1991) and Fekadu (1995), where they reported 27.3, 29.4, 44.6, 39.8 and 38.65%, respectively.

The present finding showed clinical mastitis cases with the prevalence level of 9.4% in Holstein, local zebu and jersey breeds. The clinical prevalence in this study was similar to that of Tollosa et al. (2009) who reported the prevalence of 9.5% at wolayta sodo and higher than report of Bishi (1998) who reported the prevalence of 5.3% in Addis Ababa (Nesru et al., 1997) who reported 5.3% in central Ethiopia and lower than those reported by Handera et al. (2005) with the prevalence of 16.11% in and around Workineh et al. (2002) reported 25.1% in Addis Ababa.

Sub clinical mastitis was higher as compared to clinical in the three breeds. The prevalence of sub clinical mastitis at cow level based on CMT in the present study (43.5%) was higher than the finding of Demelash et al. (2005) who reported 23% Hundera et al. (2005) who reported 36.6%, Tolloso et al. (2009) who reported the prevalence in wolyta sodo (17.5%).

The variability in the prevalence of bovine mastitis between reports could be attributed to the difference in management of the farm, breeds, season of the study, agro climactic condition or diagnostic test employed. In this study, the prevalence of mastitis as sub-clinical disease entity was higher (43.5%) than clinical forms of mastitis. Robertson (1985) concludes that sub clinical mastitis was usually far higher than clinical mastitis. In Ethiopia, the sub clinical form of mastitis received little attention and efforts have been concentrated on the

Table 2. The prevalence of mastitis based on lactation stage.

Sampled animal	Lactation stage (days)			
	1-120 (Beginning) (%)	120-240 (Mid) (%)	>240 (End) (%)	Total (%)
Infected	78 (37.5)	21 (10.3)	104 (51.2)	203 (52.9)
Non-infected	48 (26.5)	57 (31.4)	76 (41.9)	181 (47.1)
Total	126 (61.9)	78 (26.9)	180 (57.7)	384 (100)

X^2 = 26.642, P<0.05.

Table 3. Showing prevalence of mastitis in different parity group of animals.

Parity group	No. of animals examined		Prevalence (%)
	Affected (%)	Non affected (%)	Total
1 and 2 (G1)	88 (51.6)	84 (48.8)	172 (44.8)
3 and 4 (G2)	91 (58.3)	65 (41.7)	156 (40.6)
5 and 6 (G3)	24 (42.8)	32 (57.1)	56 (14.6)
Total	203 (52.9)	181 (43.1)	384 (100)

X^2 = 3.341, P-value = 0.188.

Table 4. Prevalence of mastitis with respect to different age group.

Age	Positive (%)	Negative (%)	Total (%)
Young	78(38.4)	83(45.8)	161(41.93)
Adult	99 (48.7)	64(35.3)	163(42.45)
Old	26(12.8)	34(18.8)	60(15.6)
Total	203(52.9)	181(47.1)	384(100)

X^2 = 7.496, P = 0.023.

treatment of clinical cases (Hussein et al., 1997) while the economic loss could come from sub clinical mastitis.

Age is a detrimental factor in the distribution of the diseases because at some time it is stress. It was taken into consideration and the prevalence of mastitis was measured for different age groups of lactating cows. The prevalence was found to be much higher in the young (20.3%) and adult (25.8%) age group than the older (6.8%) age group. This is statically significant with P<0.005.

The finding of this study was also assessed for breed predisposition to mastitis but no significant difference in the prevalence was detected between the three breed. It has been reported that mastitis prevalence may be influenced by some inheritable characteristic such as capacity of milk production teat characteristic and udder conformation (Abaineh, 1997). However, the insignificant difference in the prevalence of mastitis between the three breeds reported in this work needs further investigation before a satisfactory explanation is being forwarded. It is worthwhile to mention here that the indigenous zebu stocks are subjected to poor management conditions as compared to Jersey and Holstein cows.

The relationship between the prevalence of mastitis on different lactation stage was studied; the result showed significantly higher infection (p<0.05) in cow with early and late lactation than cow with mid lactation stage. This finding agreed with that of Demelash (1994) where the prevalence of mastitis is higher in the early and late lactation stage. Early stage of lactation and the period of involution of the mammary gland were the most susceptible stage with prevalence of mastitis.

The increase in the prevalence of mastitis with increasing number of lactation reported in this study coincides with result obtained by Osei (1974), Smith et al. (1985) who reported that increase in the prevalence of mastitis accompanied the increase in the lactation number. Sharf et al. (2009) reported that first lactation cows were most resistant to infection. Among the several explanation for the multifarious relationship are increase in teat potency (Murphy, 1994), in this study, the prevalence has been observed to increase up to 4^{th} parity group and decline at subsequent lactation.

With regard to the bacteriological analysis of milk sample, the work revealed that from the CMT positive milk sample the mixed bacterial isolates were the most

Table 5. The relative isolation rate of mastitis causing bacteria.

S/N	Bacterial spices	Frequency	
		No. isolated	Isolation (%)
1	*S. aureus*	136	54.4
2	*S. Dysagalatiae*	62	24.8
3	*S. intermidius*	21	8.4
4	*S. uberis*	13	5.2
5	*S. epidermides*	11	4.4
6	*S. agalactiae*	4	1.6
7	*C. pyogens*	2	0.8
8	*E. coli*	1	0.4
	Overall	250	100

prevalent than each isolated bacteria 114 (29.7%). It was reported that *Streptococcus* species together with *Staphylococcus* species were the most important causes of bovine mastitis (Blood and Radostitis, 1989). And the species of bacteria isolated *S. aureus* was most commonly isolated in clinical and sub clinical case of mastitis in this study case. In this study, *S. aureus* was the predominant pathogen involved constituting 54.4% of all isolate. The high level isolation of *S. aureus* (54.4%) in this study is related with the finding of Ahamed and Mohammed (2007) in Egypt who reported 52.5% and higher than that of Lakew et al. (2009) who reported 39.4%. This finding was not in harmony with reports of Bishi (1998) and Edwards et al. (1982) who found CNS as the predominant species from urban and peri-urban production system in Ethiopia and Bolivia, respectively.

The reason for the higher isolation rate of this organism is the wide ecological distribution inside the mammary gland and skin. In area where hand milking and improper use of drug is practiced to treat the mastitis cases, its domination has been reported by many research scholars. *S.aureus* is adapted to survive in the udder and usually establishes mild sub clinical infection of long duration from which it is shaded through milk serving as sources of infection for other healthy cows and transmitted during the milking process (Radostitis et al., 1994). Hence, the organism has been assuming a position of major importance as a cause of bovine mastitis.

On the other hand, as compared to the proportion of *Staphylococcus,* lower percentage (24.8%) of *Streptococcus* spp. (*Streptococcus dysgalactia*) was isolated. This finding corresponds to the reports made by Geressu (1989), Nesru et al. (1997), Tiruneh (1996) and Hamir et al. (1978) who reported *Streptococcus* spp. as the second major bacteria that causes mastitis and from this species of bacteria, *Streptococcus uberis* and *Streptococcus agalactia* (5.2%) and (1.6%) are next, respectively.

The isolation of *Corynebacterium* spp. was only 1%. The finding was slightly in agree with the findings of Hamir et al. (1978) who reported 1.3%. Coliform spp. with the infection rate in this study was lower as compared to the other bacterial species. *E. coli* (0.5%) out of all bacterial isolated from mastitis positive milk this finding was lower than that of Molalegne et al. (2010), Mengistu (1986) who reported (2.5%) and (3.14%) respactively. In general, the prevalence of mastitis causing agents are high. Thus, the farms should follow the key factors of mastitis program such as good herd management, teat dipping before and after milking, washing milkers hands before and after milking, preparation of clean towel for each lactating cow, milking of infected cow lastly, using dry cow therapy method and treating clinical cases at early stage.

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