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STUDY ON PREVALENCE, BACTERIAL PATHOGENS AND ASSOCIATED RISK FACTORS OF BOVINE MASTITIS IN SMALL HOLDER DAIRY FARMS IN AND AROUND ADDIS ABABA, ETHIOPIA

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ABSTRACT

A cross-sectional study was conducted with the purpose of determining the prevalence, bacterial pathogens and the associated risk factors of bovine mastitis in Small holder dairy farms in and around Addis Ababa, Ethiopia. The study was carried out on 499 dairy cows based on data collection questionnaire survey, farm visit, animal examination and California mastitis test (CMT) and isolation of the causative agent. The present study revealed abnormalities of udder as evidence of mastitis in 373(74.7%) cows. Of these 98(19.6%) were clinical and 275 (55.1%) sub clinical form of mastitis. Quarter level prevalence was 5.2% and 42.7% for clinical and sub clinical mastitis, respectively. The prevalence among successive stage of lactation was 87.2%, 65.9% and 73.1% in early, mid and late lactation, respectively. This variation was statistically significant ($P<0.05$). Parity and age were also considered as major intrinsic risk factors that influenced prevalence of mastitis. Milking practice and farm hygiene were considered as extrinsic risk factors, where owners who practiced using towel had 62.9 % infection rate and where as those that did not used were affected at a rate of 79.7%. The prevalence of cows that were managed in poor hygienic condition was 82.6%, while those managed under poor hygienic condition showed an infection rate of 59.6%, these prevalence were significantly different ($P<0.05$). Bacteriological examination of milk sample revealed the highest isolation rate of *Staphylococcus aureus* at a rate of 28.8% and *Micrococcus* shows the least percent 5%. The present study concluded that mastitis was a major health problem of dairy cows in the area which undoubtedly affect productivity of dairy industry and hence warrants serious attention.

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

INTRODUCTION

In terms of livestock population, Ethiopia has the largest population of any African country. Furthermore, cows represent the largest population of cattle production of the country (CSA, 2009). Milk produced from these animals provides an important dietary source for the majority of rural as well as considerable number of the urban and per-urban population. However; milk production often does not satisfy the countries requirement. According to the reports of (FAO, 2003), the total annual national milk production in Ethiopia ranges from 797, 9000 to 1,197, 500 metric tons raw milk equivalents. Out of the total national milk production, between 85 and 89 percent is contributed from cattle. However, this amount is by far below the national demand for milk and milk products in the country, given the considerable potential for small holder income and employment generation from high value dairy products.

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 imposes a serious problem cause reduction in milk production (Korhonen and Kaartinen, 1995) and remains one of the most economically important diseases for the dairy industry worldwide irrespective of the species of animals (Bradley, 2002). Mastitis can cause devastating effects to farmers because of the serious economic losses and the danger that the bacterial contamination of milk from affected cows may render it unsuitable for human consumption (Quinn *et al.,* 1999). Mastitis occurs worldwide among dairy animals and it has been described to have an extreme zonotic and economic impact (Al-Majali *et al.,* 2008).

Mastitis can be defined as clinical or subclinical. Clinical cases of mastitis are characterized by the presence of one or more of symptoms such as abnormal milk, udder swelling and systemic signs including elevated temperature, lethargy and anorexia (Eriskine, 2001). Sub clinical mastitis are those in which no visible appearance of changes in the milk or udder, but milk production decreases, bacteria are present in the secretion and composition is altered (Eriskine, 2001). For every case of clinical mastitis there are 20-40 times as many cases of sub clinical mastitis (Eriskine, 2001). Sub clinical mastitis in the mammary gland is detectable only

by determining high SCC in milk or by bacterial culture (Quinn *et al.* 1994).

Mastitis is a complex and multi factorial disease the occurrence of which depends on variables related to the animal, environment and pathogen (Radostits *et al.,* 2007). Among the pathogens, bacterial agent are the most common one, the greatest share of which resides widely distributed in the environment of dairy cows, hence a common threat to the mammary gland (Bradley, 2002). Although the occurrence of mastitis in Ethiopia has been reported from different parts of the country (Sori *et al.,* 2005; Kifle and Tadelle, 2008, Almaw *et al.,* 2009), regular and systematic studies of the disease 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 provide information on the prevalence of clinical and sub clinical mastitis in lactating cows, to isolate and identify the predominant etiological agents of mastitis and to assess the associated risk factors in Small holder dairy farms in Addis Ababa, Ethiopia.

MATERIALS AND METHODS

The study was conducted in Addis Ababa. Addis Ababa is located at 9.03^0 North latitude and 38.8^0 East longitudes with an average altitude of 2400 meters above sea level. It has an average annual temperature of 15.9^0C. It also receives an annual rain fall of 1089 mm or 91 mm per month with 60.1% annual relative humidity which ranges from 49% in February to 82% in July (NMSA, 2007).

The study was a cross-sectional study where lactating cows from 38 small holder dairy farms of Addis Ababa 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 80% (Gemachis, 2008) while the statistical confidence level was 95%. Accordingly, the sample size of lactating cows was determined to be 246 based on the formula but to increase precision doubling of the sample were applied, therefore the sample size was 499. In the study area, the dairy cattle managed intensively were kept in exclusive stalls and provided with supplementary diets in addition to natural pasture and agricultural byproduct. Pre-milking hygienic procedures before each milking were done in the farm while visiting the farm and milking was done manually in the entire farms during the study. Cows were allowed for udder preparation by washing the teats and the whole udder before each milking.

Data was collected using a semi-structured questionnaire with the objective of elucidating the multi factorial background of mastitis. Data collected includes, age, parity number, lactation stage, dry cow therapy, milking procedure and blind udder was also recorded. Clinical mastitis was diagnosed on the basis of visible or palpable sign of inflammation together with change in consistency and color of milk secreted. On the other hand, California Mastitis Test (CMT) was applied to all samples for screening of sub clinical mastitis and samples for bacteriological culture, according to the method described by Schalm *et al.* (1971). According to the reaction obtained the results were classified as negative (no gel-formation), trace 1, 2, 3 reaction in which one and above results are considered positive (Quinn *et al.,* 1999). The samples for isolation of major bacterial pathogens of mastitis were placed in an icebox and transported to laboratory for processing and culturing. Identification and isolation of the bacteria was done based on the identification key set by Quinn *et al.* (1999).

The data was analyzed by using STATA software version 7 (STAT, 2001). The effect of risk factors such as age, lactation stage, udder preparation and farm hygiene with possible association of the disease was analyzed using chi- square. Values were considered significant at $P<0.05$ was considered significant in all analysis.

RESULTS

A total of 499 cross-bred cows from 38 dairy farms were examined for mastitis detection and out of which 373 (74.7%) cows were found to be affected with clinical and sub clinical mastitis based on the clinical diagnosis and CMT. Likewise, CMT positive for the sub clinical mastitis were found to be 275 (55.1%) (Table1). Out of the 1898 quarter examined 98 (19.6%) quarters which belongs to 73 (14.6%) animals were found to be blind teat. Up on screening of the functional teats (1898) by CMT, a quarter of 909 (42.7%) found to be affected by sub clinical mastitis.

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

Form of mastitis	Total examined cows	Total No. affected (%)	Total examined quarter	Total No. affected (%)
Clinical	499	98 (19.6%)	1898	98 (19.6%)
Sub clinical	499	275 (55.1%)	1898	811 (42.7%)
Total	**499**	**373 (74.7%)**	**1898**	**909 (62.3%)**

In quarter prevalence of sub clinical mastitis, right rear teats (RR) showed the highest rate of infection (48.9%) followed by the left rear (LR) which is 48.1%. The overall quarter prevalence of sub clinical mastitis was 47.9% (Table 2).

Table 2: Quarter prevalence of sub clinical mastitis using California Mastitis Test

Quarter	No examined	Positive	Frequency (%)
RF	480	229	47.7
RR	474	232	48.9
LF	478	224	46.9
LR	466	224	48.1
Total	**1898**	**909**	**47.9**

Result of bacterial analysis: Milk sample of 118 quarters, which were positive for CMT were cultured for microbiological examination and 80(67.8 %) yielded bacteria. The bacterial isolation rate and their prevalence are shown on (Table 3). The predominant isolated bacteria were *Staphylococcus aureus* with isolation rate of 28.7% followed by *Streptococcus agalactiae* with isolation rate of 21.2%. *E.coli* was the third predominant isolated with isolation rate of 18.7%. *Micrococcus sp.* was the least isolate which accounts for 5%.

Table 3: Bacterial species isolated from bovine mastitis

Bacterial species	Total number of isolates	Prevalence (%)
Staphylococcus aureus	23	28.7
Micrococcus species	4	5.0
Streptococcus dysagalactie	7	8.7
Streptococcus faecalis	8	10.0
Streptococcus agalactiae	17	21.2
Pseudomonas aeroginosa	6	7.5
E.coli	15	18.7
Total	**80**	**100**

Result on Risk Factors

Intrinsic risk factors: Prevalence of mastitis related to specific risk factors were determined as the proportion of affected cows out of the total examined. As indicated in (Table 4) age, parity and lactation stage were found to be having significant difference on the prevalence of bovine mastitis ($P<0.05$). Cows at age group of young adult and adult had an infection rate of 65% and 93.2%, respectively. Higher infection rate (87.2%) was recorded during the early lactation stage as compared to mid lactation stage that accounted for 65.9% and for late lactation 73.1% also cows having greater than 5 calves were more affected than those with fewer and moderate calves (Table 4).

Table 4: The prevalence of bovine mastitis in milking cows based on stage of lactation, age and parity

Host risk factors	Total number of animal examined	Number of animals affected	Prevalence (%)	$\chi 2$	P- value
Age					
3-5 years (Young adult)	323	210	65.0	48.13	0.000
6-10 years (adult)	176	164	93.2		
Lactation stage					
Early (< 4 month)	133	116	87.2		
Mid (5-7 Month)	132	87	65.9	16.84	0.000
Late (> 8 Month)	234	171	73.1		
Parity Number					
Cow with 1-3 calf	94	43	45.7		
Cows with 2 calves	151	104	68.9		
Cows with 3 calves	102	87	85.3	77.36	0.000
Cows with 4 calves	108	96	88.9		
Cows with 5 calves	33	33	100		
Cows with 6 calves	11	11	100		

Extrinsic Risk Factors: Management factors such as husbandry system, dry cow therapy and milking practice were evaluated as risk factors that influence the prevalence of bovine mastitis. As indicated in Table 5 owners who practiced using towel after and before milking had a lesser chance of development of bovine mastitis (62.9%) than those who did not use towel (79.7%) which had significant difference on prevalence of bovine mastitis ($P< 0.05$).

The occurrence of mastitis was also assessed in relation to dry cow therapy. Cows with no dry cow therapy were affected at higher rate (95.9%) than those with dry cow therapy (69.9%) which influences significantly the prevalence of bovine mastitis (Table 5).

Hygienic standard of the farm was also assessed in relation to the prevalence of bovine mastitis (Table 5). Cow managed under poor hygienic condition had risk of contracting the disease (82.6%) than those managed in good hygienic condition (59.6%).

Table 5: The prevalence of bovine mastitis based on milking practice.

Risk factors	Total number of animal examined	Number of animal affected	% affected	χ^2	P - value
Use towel					
Yes	140	88	62.86	15.15	0.000
No	359	286	79.67		
Dry cow therapy				28.08	0.000
Yes	402	281	69.9		
No	97	93	95.88		
Hygiene					
Good	166	99	59.64	31.1	0.000
Poor	333	275	82.58		

DISCUSSION

The present study showed an overall prevalence of 74.7 % as determined by the CMT and clinical examinations of the udder and this finding is comparable with that of Mekbib *et al.* (2011) who reported 71.0%. But it was higher than the finding of Delelesse (2010) and Sori *et al.* (2005) who reported 44.1% and 52.78%, respectively. In the same manner, the prevalence rate for clinical mastitis obtained in this study area (19.6%) was comparable with the finding of Alemnew (1999) (21%) in Modjo state owned dairy farm, Ethiopia. But the present finding was higher than the report made by Delelesse (2010) (10.3%) around Holeta area, Ethiopia; but the present finding was lower than the reports of Workineh *et al.* (2002) (25.1%) in Addis Ababa, Ethiopia, but by far higher than the finding of Enyew (2004) (3.9%) from Bahir Dar, Ethiopia. As mastitis is a complex disease involving interactions of various factors such as managemental and husbandry, environmental conditions, animal risk factors, and causative agents, its prevalence will vary (Radostitis *et al.,* 2007).

In case of sub clinical mastitis the prevalence rate at cow level (55.1%) obtained in this study was comparable with the finding reported by Biffa (1994) (54.4%) and (Bedada and Hiko, 2011) (55.8%). However, very recently lower prevalence in the range of 14.8%-36.67% (Enyew, 2004; Sori *et al.*, 2005; Almaw *et al.*, 2009; Delelesse, 2010; Moges *et al.,* 2011) and prevalence as high as 62.9%-95% has been reported (Dego and Tarek, 2003; Bedada and Hiko, 2011; Byarugaba, 2008; Kifle and Tolosa, 2008. Since, environmental factors play significant role, the prevalence of sub-clinical mastitis varies in dairy animals (Radostits *et al.*, 2007).

In this study sub clinical mastitis has been found to be higher than clinical mastitis. This could be attributed to the 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). A similar observation of the dominance of subclinical mastitis was observed by several studies (Workineh *et al.*, 2002; Dego and Tarek, 2003; Sori *et al.*, 2011).

Quarter prevalence of mastitis (62.3%) found in this study was comparable with the finding of Kifle and Tolosa (2008) who reported quarter prevalence rate of 63.1%, but higher than the report made by Bachaya *et al.* (2011) in Pakistan, Zelalem (2001) in Ethiopia, Fadlelmoula *et al.* (2007) in Germany, who reported 35.25%, 15.92%, and 27.57%, respectively. As compared to the others the right rear quarters were affected with the highest infection rate (48.9%). The left rear quarters were the second with an infection rate of 48.1%. This might be due to the high production capacity of the hind quarters (Radostitis and Blood, 1994) and the high chance of getting fecal and environmental contamination (Sori *et al.*, 2005).

The result obtained from bacteriological analysis of the samples revealed that from the total of 118 quarter sample, 80 (67.8%) were bacteriologically positive which is higher than Zelalem (2001) who reported proportions of 18%. This may be due to bactericidal properties of inflammatory udder secretions. In this study the predominant organisms isolated from clinical and sub clinical mastitis to be *Staphylococcus aureus* followed by *Streptococcus agalactiae.* 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). Radostitis *et al.* (2007) asserted that *S. aureus* 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 transmission to healthy animals mainly during milking. The least identified isolate was

Micrococcus spp. as was observed by Mekbib *et al.* (2010) in Central Ethiopia.

This study revealed the prevalence of mastitis to be affected significantly with lactation stage. Early lactation stage had higher relative prevalence (87.2%) than late (73.1%) and mid (65.9%) lactation stage; the difference is statistically significant ($p<0.05$). This result was in close alignment with reports made elsewhere (Alemnew, 1999; Delelesses, 2010; Moges *et al.*, 2011). Absence of dry cow therapy regime could possibly be the major factor contributing to high prevalence at early lactation and early infection associated with delayed diapedesis of neutrophils in to the mammary gland (Schalm *et al.,* 1971). In the present study the prevalence of mastitis was higher in adult cows (93.2%) than young adults (65%) and the higher prevalence of mastitis with increasing age has been reported (Moges *et al.*, 2011). Radostitis *et al.* (2007) have explained that older cows have largest teats and more relaxed sphincter muscles, which increase the accessibility of infectious agent in the cows' udder. Cows with many calves were greater at risk than those of cows having moderate and few calves. Similar finding was reported by Moges *et al.* (2011).

Cows that were not treated during dry period were more affected than those treated and there was statistical difference between treated and untreated groups ($P<0.05$). This difference could be associated with the low bactericidal and bacteriostatic quality of milk during dry period. Moreover, the capacity of the quarter to provide phagocytic and bactericidal activity generally diminishes during dry period (Paape and Miller, 1996). Milking practice had a significant influence on prevalence of bovine mastitis. In this study, owners who didn't use towel before and after milking found to have high prevalence of mastitis than owners who used towel. Radostitis *et al.* (2007) documented that udder preparation both before and after milking influence the rate of mastitis.

The present study concluded that mastitis was a major health problem of dairy cows in the area and undoubtedly will have an adverse effect on productivity of dairy industry and hence warrants serious attention. Good record keeping practice on the general herd health of dairy farms, adequate housing with proper sanitation and ventilation should be regularly 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 techniques 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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