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Prevalence and Identification of Predominant Causative Agents of Bovine Mastitis in Boke Woreda of Western Hararghe Zone, Oromia Regional State, Ethiopia

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Research Article

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ABSTRACT

A cross sectional study was carried out from November 2010 to April 2011 at Boke district, Western Hararghe zone of Oromia regional state to determine the prevalence and isolate the major bacterial agents that involved in bovine mastitis. The study was conducted on 384 local zebu lactating cows which were examined through clinical examination, CMT test and microbiological examination. From the 1536 quarters of 384 cows examined, 98(25.5%) were positive on CMT bases for mastitis at cows level and 157(10.2%) at quarter's level. Out of these, 80 (20.8%) subclinical and 18(4.7%) clinical at cow level were infected. whereas127 (8.3%) subclinical and 30(2%) clinical

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at quarters level. 6(1.6%) and 12(3.1%) were acute and chronic in cow level and 9(.6%) and 21(1.4%) were acute and chronic in quarter level respectively. Out of CMT positive animals, 80(20.8%) in cow level and 108(7%) in quarter level were cultured and positive for mastitis causal agents. The 1480 (96.4) quarters were functional and the rest 56(3.6%) were blind. 26(7%), 9(2.3%) and 4(1%) animals had single, double and three blind teats respectively. From CMT positive cases, 108 pathogens were isolated. The most prevalent bacterial organisms isolated were *Staphylococcus* species (19%) following by Streptococcus species (18%) and Enterobacteriacae (16%).The predominant bacterial species from the isolated (*Staphylococcus aureus, E.coli*, *Streptococcus agalactiae*) were the highest number of isolates. Risk factors analysis revealed that the age ($P<0.05$), parity ($P<0.05$) and udder hygiene condition ($P<0.05$) were highly significant for the prevalence of mastitis. Therefore treating clinically infected cows, regular screening for early detection of subclinical mastitis were important in reducing the prevalence of mastitis in the study area.

INTRODUCTION

Rapidly growing demand for livestock products worldwide is brought about by increasing human pressure, growing income and urbanization[1]. The world's 220 million dairy cows produce approximately 430 million tons of milk per year[2]. In sub Saharan African countries, livestock plays a crucial role both for the national economics and the livelihood of rural communities[3]. The large majority of milk producers in the tropics and sub tropics of Africa are small holders who produce milk for their own requirements[2]. Ethiopia holds a large potential for dairy development[4]. The country has the largest livestock population of any African country. Livestock represents a major national resource and form an integral part of the agricultural production system[5].The country currently manages the largest livestock population estimated at about 48 million cattle[4]. Cows represent the largest proportion of cattle population of the country. Food and Agricultural Organization estimated that 42% of the total cattle herds, for the private holdings are milking cows[6].

The total annual milk production is estimated at 797,900 to 1,197,500 metric tons of raw milk, out of which between 85% and 89% is derived from cows[7]. However, compared to other African country, Ethiopia consumes less dairy cattle products. Per capital consumption of milk in Ethiopia is as low as 17 kg per head while the average figure for Africa is 26 kg per head[8]. Given the considerable potential for small holder income and employment of the dairy sector in Ethiopia can contribute significantly to poverty alleviation and nutrition in the country[9]. Nevertheless the quality and the quantity of milk in the country deteriorate because of various causes[10]. The incriminated biological causes include the low genetic potential of the animals, poor nutrition and prevalence of disease[11,12]. In the livestock development policy to improve the capital milk consumption, improvement of the genetic potential of the indigenous zebu through breeding with high grade exotics was included[13].

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Milk is one of the most important foods of human beings. It is universally recognized as complete diet due to its essential component[14]. It is a very nutritional food that is rich in carbohydrates, proteins, fats, vitamins and minerals[15]. The quality of milk lowered by a number of factors such as adulteration, contamination during and after milking and the presence of udder infection. The udder infection has occurred if the invading pathogen causes an inflammatory response and loss of potential milk production in the affected quarter of gland. Disease of the mammary glands known as mastitis is among the various factors contributing to reduced milk production[16]. It is the most economically important disease in dairy milk production worldwide[17-19]. Average costs of clinical mastitis cases can range from 100 to 200 USD/case/year and 40 to 100 USD/herd/years[20]. According to the study carried out in England and Wales from 1979 to 1982, the average cost of a case of mastitis due to antibiotic used, milk discarded, reduction in quantity and quality of milk produced by a cow was estimated 60 pound for each case. Ethiopian farmers' especially small holders were unaware of the invisible loss from sub clinical mastitis[21] and were also true in Tanzanian and Ugandan farmers[22,23].

The economic losses from mastitis in the urban and peri urban areas of Addis Ababa, Ethiopia to be USD 58 per cow per lactation[24]. Economic losses from clinical and sub clinical mastitis in Addis Ababa milk shed to be also approximately 270 Ethiopian birr per lactation[25]. The disease is characterized by physical, chemical and bacteriological changes in the milk and pathological changes in the milk include discoloration, presence of clots and presence of large number of leukocytes[26]. As with most infectious disease, mastitis risk factors depend on three components: exposures to microbes, defense mechanism of host, and environmental and manage mental factors[5]. This disease can have infectious or noninfectious etiology, and the infectious pathogen is the most important ones that frequently due to infection by one and/or the other pathogens, such as bacteria, viruses, mycoplasma, yeasts and algae[27- 31]. The most causative agent of mastitis is bacterial origin and a few of species of bacteria account for most cases, such as *E. coli*, *S. aureus*, *St.Uberis*, *St. dysgalactiae* and *St.agalactiae*[30, 32-35]. The most common infectious agent implicated as cause of mastitis in cattle are *St. agalactiae* and *S. aureus*[30] whereas, environmental mastitis is associated with coli forms and environmental *streptococci* that are frequently found in the cows Environment[26,36].In Ethiopia, mastitis has long been known[37-41].Even though the disease of mastitis has been known locally, it has not been studied systemically making information available on the prevalence of the disease and associated economic loss inadequate[5]. Therefore this study is carried out in Eastern part of Ethiopia for the following objectives:

1. To assess the prevalence of bovine mastitis in the study area
2. To isolate and identify the predominant etiological agents responsible for the occurrence of the disease
3. To determine the potential risk factors associated with the disease.

MATERIALS AND METHODS

Study area

The study was conducted from the beginning of November 2010 to the end of April 2011 in selected peasant associations of Boke district of Western Hararghe found in Oromia regional state. Boke district is located at 385 km east of the capital city Addis Ababa. The district has 22 peasant associations, total coverage of 76,744 hectares with human populations of 127, 982. This district has an average daily temperature ranges from 15°-28°C, annual

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rain fall ranges from 5oo mm-1200 mm and its altitude ranges between 1600 to 1800 meters above sea level. The district has 95,686 cattle, 47,944 goats, 3145 sheep, 15,604 donkeys, 63 mules, 36 horses, 782 camels and 38,313 poultries. The total population of the district according to BWBA 2009 is 201,573.

Study animals

The target animals were the local zebu lactating dairy cow managed under extensive farming system in the selected peasant associations.

Study design

The study was cross-sectional to determine the prevalence of bovine mastitis in the study area. Age of the animals was determined from birth records and dental characteristics[42] and categorized as young adults (>3 to 6 years), adults (>6 to 10 years), and old (>10). Stage of lactation was categorized as early (1st to 4th month), mid (4th to 8th month), and late (8th month to the beginning of dry period). Parity was categorized as few (below 3 calves), moderate (4-7 calves) and many (>7 calves)[43]. The parity was classified in to two groups. Those with parity number 1-3 and those with parity number greater than 3. The house hygiene was described as good if the floor is made of concrete, it give comfort for the cows and easy to clean, otherwise as poor if the floor is muddy and cumbersome for cleaning.

Sample size determination

To determine the sample size, the expected prevalence to be 50%. The desired sample size for the study was calculated using the formula given by Thrufield[44] with 95% confidence interval and at 5% absolute precision.

$$\frac{1.962\ Pexp\ (1\text{-}Pexp)}{d2}$$

Where; Pexp=expected prevalence, d2=desired absolute precision and n=required sample size. Accordingly 384 total samples were calculated to perform the study.

Data collection

Questionnaire survey: Data collection was conducted to obtain appropriate information about the study animals to evaluate risk factors on the occurrence of mastitis. The data include (whether they wash their hand, udder of the lactating cows before and after milking, whether they strip the first streams of milk on ground or not), parity number, age of the cow, lactation stage and hygiene of milking process. Presence of lesions on the skin of udder, teat and milk abnormalities (injuries, blindness and swelling), and clinical state (acute, chronic) were recorded. Strict aseptic procedures for the collection of milk samples were used.

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Physical examination: Visual observation and palpation of the mammary gland and macroscopic examination of milk was undertaken in the study area. The cows were smaller in size with small udder and short teats. The average daily milk production from individual cows was relatively law (4 to 5 liters). Cows were allowed to dry off at late lactation period by cessation of milking. During physical examination clinical cases was diagnosed on the basis of visible sign of inflammation such as warm, swollen and pain up on palpation which have been considered as acute clinical mastitis while miss shaped, atrophied, hard and fibrotic quarters were considered as chronic mastitis. The amount of none functional teats were also determined.

California mastitis test: The CMT is reliable screening test used for detecting subclinical mastitis that can be easily used at the cow side. As quirt of milk, about 2 ml from each quarter was placed in each of four shallow cups in CMT paddle and then an equal amount of three percent CMT reagent was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane for 15 seconds. The California mastitis test was carried out according to the method described by Quinn[45].

Data management and analysis: Data was entered into Microsoft excel and analyzed using SPSS software program. Descriptive statistics were used to summarize and present the data generated from the study. The prevalence of mastitis was calculated using percentage values and effects of specific variables on prevalence of mastitis were analyzed by chi square statistical analysis technique using appropriate computer program.

RESULTS

Overall prevalence

From the 384 cows examined, 98 (25.5%) were positive on CMT bases for mastitis at cows level and 157 (10.2%) at quarters level. Out of these, 80 (20.8%) was found to be subclinical and 18 (4.7%) clinical at cow level whereas127 (8.3%) for subclinical and 30 (2%) clinical at quarters level (Table1). Out of CMT positive animals, 80 (20.8%) in cow level and 108 (7%) in quarter level were cultured and positive for mastitis causal agents. Among 1536 quarters 1480 (96.4) were functional and the rest 56(3.6%) were blind while 26 (7%), 9 (2.3%), and 4 (1%) animals had single, double and three blind teats, respectively (Table 1).

Table 1. Prevalence of mastitis based on CMT and culture result.

Observational level	Number of observed	Prevalence		
		CMT positive (%)	Culture (%)	Blind teats (%)
Cow level	384	98 (25.5%)	80 (20.8%)	35 (9%)
Quarter level	1536	157 (10.2%)	108 (7%)	56 (4%)

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Prevalence of clinical mastitis

Eighteen (4.7%) cows and 30 (2%) quarters were clinically infected and 6 (1.6%) and 12 (3.1%) were acute and chronic in cow level and 9 (0.6%) and 21(1.4%) were acute and chronic, respectively. Different bacterial species were isolated from all clinical cases (Table2).

Prevalence of sub clinical mastitis

Eighty (20.8%) cows were infected with subclinical mastitis and 127 (8.3%) quarters were found to be positive for mastitis on CMT basis. Major bacterial causative agents of mastitis were isolated from 62 (16.1%) and 78(5.1%) in cows and quarter level, respectively (Table2).

Table 2. Prevalence of subclinical and clinical mastitis.

Observational level	Observation Number	Prevalence			
		Subclinical (%)	Clinical (%)	Acute (%)	Chronic (%)
Cow level	384	80 (20.8%)	18 (4.7%)	6 (1.6%)	12 (3.1%)
Quarter level	1536	108 (7%)	30 (2%)	9 (0.6%)	21 (1.4%)

Etiological agents

From CMT positive cases, 108 pathogens were isolated using primary and secondary bacterial isolation and identification tests. The most prevalent bacterial organisms isolated were *Staphylococcus* species (19%) following by *Streptococcus* species (18%), Enterobacteriacae (16%) and others. The major predominant bacterial species isolated were found to be *S. auereus*, *E. coli* and *St. Agalactiae*. The type, number, and proportion of bacterial isolates were presented (Table 3).

Table 3. Microorganisms causing mastitis isolated from milk samples of study area.

No	Pathogens	Frequency	Percentage (%)
1	*Staphylococcus aureus*	16	14.8
2	*Escherichia coli*	14	13
3	*Streptococcus agalactiae*	11	10.2
4	*Streptococcus dysgalactiae*	9	8.3

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5	*Pasteurella haemolytica*	9	8.3
6	*Staphylococcus hyicus*	8	7.4
7	*Bacillus cerus*	7	6.5
8	*Enterobacter*	7	6.5
9	*Staphylococcus epidermidis*	6	5.6
10	*Streptococcus uberis*	6	5.6
11	*Pasteurella multocida*	6	5.6
12	*Klebsiella*	5	4.6
13	*Protues*	3	2.7
14	*Streptococcus bovis*	1	0.9
Total		108	100

Risk factors associated with mastitis

The statistically analyzed data summarized in Table 4 revealed the presence of significant effect of different factors on prevalence of mastitis. The risk factors identified were age, parity, stage of lactation, hygiene, position of the quarters.

Table 4. Association of risk factors with prevalence of CMT positive mastitis at cow level.

Risk factors	Groups	Number of observation	CMT positive (%)	Chi^2 (x^2)	P-value
Parity	1-3	333	65(19.5)	26.709	0.000
	>3	51	33(64.7)		
Age	Young	179	15(8.4)	56.863	0.000
	Adult	184	72(39.1)		
	Old	21	11(52.4)		

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Lactation stage	Early Mid Late	203 96 85	59(29) 23(24) 16(19)	0.565	0.754
House hygiene	Good Poor	44 340	8(18.2) 90(26.5)	1.408	0.235
Quarter position	Front Hind	768 768	60(7.8) 97(12.6)		
Washing hand & udder before milking	Yes No	123 261	12 (9.8) 86(33)	28.794	0.000
Striping the first stream on ground	Yes No	36 348	10(28) 88(25.3)	0.106	0.744

DISCUSSION

The overall prevalence of bovine mastitis in extensive management based on CMT was 25.5%. This finding is in close agreement with Nessru[46] who reported a prevalence rate of 25% at different farms in and around Addis Ababa at cow level. However, this result was found to be lower than the reports of previous finding in Dire-Dawa, Eastern Ethiopia by Darsema[47] with a rate of 36.9% and 38.6% prevalence in the Chaffa Valley in Northern Ethiopia by Fekadu[16]. The prevalence of subclinical (20.8%) and clinical (4.7%) mastitis at cow level in this study was still lower than the results reported by Bishi[25] who recorded 30.2% for subclinical and 5.5% for clinical and by Mungube[39] as 6.6% for clinical mastitis in Addis Ababa. The difference in prevalence described above might be due to breed type, environmental hygiene condition, and type of faming system of the study areas. Frese[48] found that large farms located in urban areas within limited operational space are associated with hygiene risk factors

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that have significant contribution to the emergence of mastitis problem. Small holder farms are generally more efficient than large scale farms in most urban and per urban areas[49].

The prevalence of subclinical mastitis at cow level obtained in this study (20.8%) was higher than the finding reported by Butsuamlak[50] which was 15.25% in and around (12.2%) [51]. A relatively similar findings were reported by Moges[52] with a rate of 21% in Modjo state owned dairy farm, 21.3% in Soddo by Shimelis[53] and Getahun[54] who reported 22.3% in small holder dairy farms in the central highland of Etiopia. Among the major bacterial pathogens isolated from milk samples in this study, *Staphylococcus aureus* was found to be the predominant species (15%). This finding is in agreement with the reports of Workneh, Nessru, vaarst and Envoldsen[37,46,55] who indicated the *S. aureus* as the primary species in bovine mastitis[12]. This might be the reason that *Staphylococcus* is well adapted to survive in the udder and usually establishes a mild subclinical infection of long duration from which it is shed into milk and facilities transmission to healthy animals mainly during milking procedure[26].

In this study, prevalence of mastitis increased significantly with parity number (Table 4). Animals with parity greater than 3 were more affected (64.7%) than those with parity from 1-3 (19.5%). The same pattern of effect of parity was reported by Duniel and Schukken[55-57]. It has been indicated that younger animals have a decreased susceptibility to mastitis through a more effective host defense mechanism while older cows, especially after four calving are more susceptible[56,57].

Even though house hygiene has risk factor on the occurrence of the disease in this study, it was found to be insignificant. This might be due to the fact that the extensive management system has less exposure to contagious organisms since animals getting fresh open housing relatively every other day. Housing conditions that provide nutrients to bacteria, like damp and wet conditions will promote greater exposure of cows' teat to pathogens and result in higher incidence of mastitis. In the present study, washing udder and hands before milking were found to have significant effect on the prevalence of sub-clinical mastitis (3.1%)(Table 4). A similar finding was reported by Dorgent[58] that udder preparation both before and after milking influences the rate of mastitis in a given herd. This finding is also in agreement with the report of Esa[59] which has indicated that intramammary infection can be transferred from cow to cow through dirty towel and milers' hands during cleaning.

CONCLUSIONS AND RECOMMENDATIONS

The high overall prevalence of bovine mastitis observed in this study area revealed as mastitis is one of the major disease problems in dairy cows. The prevalence of subclinical mastitis at cow level showed a relatively higher prevalence rate as compared to clinical mastitis. The most predominant bacterial pathogen responsible for the high prevalence in both clinical and subclinical mastitis was found to be *S. aureus* followed by *E .coli* and *St. Agalactiae*, respectively. Parity number, age of the cow and washing both hand and udder before milking were found to have a significant association with the prevalence rate of the disease. Inadequate hygienic condition of the dairy housing system, lack of proper attention to health and management practice of dairy industry, absence of awareness among farmers/ farm owners about the disease and lack of implementation of mastitis control program might be the other important predisposing factors for the high rate of the disease in the area.

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Based on the above remarks, the following recommendations are indicated:

1. There should be a prevention and control strategic plan which needs to be implemented in the study area.
2. Training and awareness creating about the disease management practices like milking and hygienic conditions and also the economic significance of the diseases should be delivered to the farmers and to the stockholders and the administrative body of study area.
3. Further studies should be conducted to determine the other importance potential risk factors associated with the occurrence of bovine mastitis.

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