Bovine Mastitis: Prevalence, Risk Factors and Major Pathogens in Dairy Farms of Holeta Town, Central Ethiopia

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Abstract

A cross sectional study was carried out from November 2008 to April 2009 to estimate prevalence of mastitis and to see associated bacterial pathogens in lactating dairy cows in Holeta town. A total of 107 cross bred milking cows were tested using California Mastitis Test (CMT). Prevalence of mastitis at cow level was 71.0% (76/107), out of which 22.4% (24/107) and 48.6% (52/107) were clinical and subclinical, respectively. The quarter level prevalence was 44.9% (192/428); from this the clinical and subclinical forms were 10.0% (43/428) and 34.8% (149/428), respectively. Out of the 43 quarters with clinical cases, 31 had blind teats while 12 of them revealed active cases of mastitis. Samples from all 12 active clinical cases and 90.0% (134/149) of the CMT positive subclinical quarters were found to be culture positive. From 146 culture positive samples, a total of 153 bacteria were isolated, the most prevalent being *S. aureus* (47.1%) followed by Coagulase negative *Staphylococcus* (CNS) (30.1%). Other bacterial isolates included *Streptococcus* (7.2%), *E.coli* (4.6%), *Micrococcus species* (3.3%), *Klebsella pneumonae* (3.3%), *Enterobater aerogen* (1.3%), *Corynebacterium species* (2.0%) and *Bacillus* (1.3%). Risk factors analysis revealed that prevalence significantly differed with the age ($P < 0.05$), parity ($P < 0.05$) and udder hygiene condition ($P < 0.03$). Thus, prevalence was relatively higher in adult cows (OR = 2.0; 95% CI = 1.15, 3.64), cows with moderate calves (OR = 2.4; 95% CI = 1.6, 3.6), cows with injured teat (OR = 7.7, 95%CI = 0.9, 64.1) and cows with unwashed udder (OR = 2.3, 95% CI = 0.8, 6.4) than those corresponding animals. In conclusion, this study revealed the importance of mastitis and associated bacterial pathogen in the study area.

Key words: Bovine mastitis; prevalence; risk factors; major pathogens; central Ethiopia

Introduction

Dairy production is a biologically efficient system that converts feed and roughages to milk (Yohannes 2003). Milk is a very nutritional food that is rich in carbohydrate, proteins, fats, vitamins and minerals. The increase in human population, accessibility to technology input, high demand for animal products and purchasing power in urban center had helped the urban and per urban dairy farm in the country to flourish (Yoseph et al., 1998). FAO (2003) estimated that 42% of the total cattle herds, for the private holdings are milking cows. However, milk production often does not satisfy the country's milk requirements due to a multitude of associated factors. Mastitis, known to be a complex and costly disease of dairy cows, that results from the interaction of the cow and environment including milking machine and microorganism (Azmi et al., 2008).

Mastitis has been known to cause a great deal of loss or reduction of productivity to influence the quality and quantity of milk yield and to cause culling of animals at an unacceptable age (Vaarst and Envoldsen 1997). Moreover, due to its latent form, heavy financial losses and great nutritional and technological impacts can be resulted. Because valuable components of the milk like lactose, fat and casein are decreased while undesirable components like ions and enzymes are increased and making the milk unfit for processing technology (Girma 2001; Shitandi 2004). Many infectious agents have been implicated as cause of mastitis in cattle the most common organisms being Streptococcus agalactiae and S. aureus (CSA 2004), whereas, environmental mastitis is associated with Coliforms and environmental Streptococci that are frequently found in the cows environment (Quinn et al., 2002; Radostits et al., 2000).

Mastitis as a disease, especially the subclinical form, has received little attention in Ethiopia; efforts have only been concentrated on the treatment of clinical cases (Girma 2001). Some studies have been conducted so far on the prevalence and the major

cause of bovine mastitis in the country (Workineh et al., 2002; Biffa et al., 2005; Sori et al., 2005). The current study also attempted to quantify the problem at cow and quarter level, which could potentially indicate the importance of the problem. Therefore, this study was designed to estimate the prevalence of clinical and subclinical mastitis in lactating dairy cow, to elucidate the associated risk factors and to isolate and characterize the major bacterial pathogens from milk samples of mastitic cows.

Materials and Methods

Study Area:

A cross sectional study was conducted in the selected dairy farms in Holeta town which is located at 40km west of Addis Ababa and at an elevation of 2400 m.a.s.l in the central Ethiopia. The area is characterized by mild subtropical weather, with average minimum and maximum annual temperatures of 6.3^{0}C and 22.1^{0}C, respectively. The area also experience bimodal rain fall pattern with a long rainy season extending from July to September while the short rainy season extends from March to April (CSA 2004).

Study Animals and Husbandry Practices:

Study animals were cross breed lactating dairy cows found in the town. The cows were managed under a small scale and semi-intensive management system. They were often provided with some supplementary diet in addition to the natural pasture and agricultural by-products.

Sample size and Sampling methods:

The sample size was determined based on the existing actual cluster of 85 small holder dairy farms in the town. The sampling frame from the study site indicated that those were small holder dairy farms having average two to three lactating cows in the sampling frame. Due to various inconveniences it was not possible to include all the farms and 50 dairy farms were randomly selected and included in the study. Therefore, all the lactating cows (107) in the selected farms were considered for the study.

Study Methodology

Detection of Mastitis:

Mastitis was detected using the California Mastitis Test (CMT) and results of clinical inspection of the udder (Quinn 1999).

Physical examination of the udder:

The udder was first examined visually and then through palpation to detect possible fibrosis, cardinal signs of inflammation, visible injury, tick infestation, atrophy of the tissue and swelling of the supra-mammary lymph nodes. Rectal temperature of those cows with clinical mastitis was taken to check systemic involvement. Information related to the previous health history of the mammary quarters and cause of blindness was obtained from case record sheets when available and by interviewing the farm owners when not. Viscosity and appearance of milk secretion from each mammary quarter were examined for the presence of clots, flakes, blood and watery secretions.

California Mastitis Teat (CMT):

Subclinical mastitis was diagnosed based on CMT results and the nature of coagulation and viscosity of the mixture (milk and CMT reagent), which show the presence and severity of the infection, respectively (Harmon 1994). Before sample collection for bacteriological examination, milk samples were examined for visible abnormalities and were screened by the CMT according to Quinn et al., (1999). From each quarter of the udder, a squirt of milk sample was placed in each of the cups on the CMT paddle and an equal amount of 3% CMT reagent was added to each cup and mixed well. Reactions were graded as 0 and Trace for negative, +1, +2 and +3 for positive (NMC 1990; Quinn et al., 1999).

Bacteriological examination of milk samples

Preparation of udder and teats:

The udder, especially the teats were cleaned and dried before milk sample collection. Dust, particles of bedding and other filth were removed by brushing the surface of the teats and udder with a dry towel. The teats were washed with tap water and dried. Then the teats were swabbed with cotton, soaked in 70% alcohol (NMC 1990). To prevent recontamination of teats during scrubbing with alcohol, teats on the far side of the udder were scrubbed with alcohol first, then those on the near side.

Sample collection, handling and storages:

Milk samples were collected by a standard milk sampling techniques (NMC 1990). To reduce contamination of the teat ends during sample collection, the near teats were sampled first followed by the far once. Approximately 10 ml of milk were collected in to a sterile test tube after discarding the first 3 milking stream. Then samples were placed in racks for ease of handling and transported in an ice to the laboratory and stored at 4^{0}C for a maximum of 24 hour until inoculated on a standard bacteriological media (Biru 1989; NMC 1990).

Bacteriological isolation and characterization:

Milk samples were bacteriologically examined according to the procedures employed by Quinn et al. (1999). In refrigerated milk samples, bacteria may be concentrated in the cream layer and held with in clumps of fat globules (NMC 1990). Hence dispersion of fat and bacteria was accomplished by warming the samples at 25^{0}C for15 minutes and shake before plating on a standard bacteriological media. A loopful of milk sample collected from each infected quarter was inoculated separately on to MacConkey agar and blood

agar base enriched with 7% defibrinated bovine blood. The inoculated plates were then incubated aerobically at 37^{0}C for 24 to 48 hours. When growth was not observed after incubation for 24 to 48 hours, the quarter's milk sample was reinoculated on an enriched tryptone-soya broth to amplify the bacterial growth. Identification of the bacteria on primary culture was made on the basis of colony morphology, hemolytic characteristics, Gram stain reaction including shape and arrangements of the bacteria, catalase and O-F tests. Staphylococci were identified based on Catalase test, growth characteristics on Mannitol salt agar and purple agar and tube coagulase test. Identification of Streptococci was made according to growth characteristics on Edwards media, Catalase test and hydrolysis of esculin. Gram negative isolates grown on MacConkey agar were identified based on growth characteristics on MacConkey agar, Oxidase reaction, Catalase test, triple sugar iron (TSI) agar ,the "IMViC" (indole ,methyl red, Voges-Proskaur, and citrate) test (Quinn 1999).

Data Collection and Analysis:

All the collected data including type of husbandry system, age, parity, udder and milk abnormalities (injuries, blindness, swelling, milk clots and other abnormal udder secretion, etc.) were recorded. Depending on clinical inspection and CMT results cases were categorized as either positive or negative; positive cases were further categorized as clinical and subclinical mastitis. Age of the study animals was determined from birth records and categorized as young adults (= 3 to 6 years), adults (> 6 to < 10 years), and old (=10 yrs). Parity was also categorized as few (with < 3 calves), moderate (4-7 calves) and many (>7 calves). Data related to previous history of the mammary quarters and causes of blindness were obtained from clinical records of the farm and interviews with the owner of the farms. The data were recorded in Microsoft Excel spread sheet for statistical analysis. Logistic regression was used to see the association of the potential risk factors with occurrence of mastitis using Stata 9 statistical software. The degree of association between risk factors and the prevalence of mastitis were analyzed using odds ratio (OR). In all the analysis, the level of significance was set at 5%.

Results and Discussion

Prevalence at cow and quarter level: Mastitis prevalence at cow level was 71.0% (76/107), out of which 22.4% (24/107) and 48.6% (52/107) were clinical and subclinical, respectively. The quarter level prevalence was 44.9% (192/428); from this the clinical form was 10.0% (43/428) and the subclinical was 34.8% (149/428). Out of the 43 quarters with clinical cases, 31 had blind teats while 12 of them revealed active cases of mastitis showing visible sign of inflammation on the udder and changes were also observed on milk. Samples from all 12 active clinical cases and 90.0% (134/149) of the CMT positive subclinical quarters were found to be culture positive.

Culture Result:

The collected milk samples from 149 CMT-positive subclinical cases and 12 clinical cases were cultured; and accordingly 134 (90%) of 149 cases and all the 12 clinical cases were found culture positive. Therefore, a total of 146 culture growths were observed (Table 2). From this 146 growth, 153 bacteria of 8 genera were isolated. About 95.2% of the milk samples (139/146) grew only one type of bacteria while 7 (4.%) of the samples were found to be mixed infection. The results of various bacterial species isolated from the clinical and subclinical cases are shown in Table 3. The most prevalent mastitis causing pathogen in this study was Staphylococcus, of which the predominant species were hemolytic, coagulase positive S. aureus, representing 47.1%, followed by Coagulase Negative Staphylococcus (CNS) 30.1%. Other bacterial isolates were Streptococcus species, Micrococcus species, *Klebsiella pneumonae, E.coli, Corynebacterium species, Enterobacter aerogen* and *Bacillus species* with decreasing order of frequency. Most Staphylococcal species were isolated from subclinical cases. S. aureus account for 3.9% and 43.1% from clinical and subclinical mastitis cases, respectively; whereas, CNS was isolated from clinical and subclinical mastitis at the rate of 1.3% and 28.8%, respectively.

Based on the bacteriological isolates, the infection was higher in left rear (40.2%) and right front (41.1%) quarters than in the remaining two quarters. However, the overall difference was not statistically significant (p>0.05). Among the different bacteria isolated from infected quarters, *Staphylococcus aureus*, coagulase negative Staphylococcus (CNS), *Streptococcus species*, *Bacillus species* and *Enterobacter aerogen* were found only in semi-intensive farming system. Other bacterial isolates, Micrococcus species, *Klebsiella pneumonae*, *E.coli* and *Corynebacterium species* were isolated from cows of small scale dairy farms only.

Analysis of cows (intrinsic) factors:

The prevalence of mastitis showed statistically significant difference among age group ($p < 0.05$) and parity ($p < 0.05$). Accordingly, the likelihood of the mastitis was two times more in adult cows (OR = 2.0; 95% CI = 1.15, 3.64) than younger cows. Cows with moderate calves (4 to 7 years) were seen to be more affected (OR = 2.4; 95% CI = 1.6, 3.6) than cows with lower parity (Table 4).

Analysis of extrinsic risk factors:

Table 5 shows the association between the occurrence

Table 1: Interpretation for the California Mastitis Test

CMT score	Interpretation	Visible reaction	Total cell count
0	Negative	Milk fluid is normal	0-200,000 (0-25% neutrophils)
T	Trace	Slight precipitation	(1.5-5) x 105 (30-40% neutrophils)
1	Weak positive	Distinct precipitation but not gel formation	(4-15) x 105 (40-60% neutrophils)
2	Distinct positive	Mixture thickens with gel formation	(8-50) x 105 (60-70% neutrophils)
3	Strong positive	Strong gel that is cohesive with a convex surface	>5,000,000(70-80% neutrophils)

Source: Quinn et al. (1999)

of mastitis and the udder hygiene condition indicating that mastitis was significantly (P = 0.03) higher in cows with poor udder hygiene and cows with injured teat (P = 0.041) than regularly washed cows and cows free of injury. Thus, there was more likelihood of mastitis occurrence in poor udder hygiene cows (OR = 2.3, 95%CI = 0.8, 6.4) and cows with injured teat (OR = 7.7, 95%CI=0.9, 64.1) when compared to regularly washed cows and cows with uninjured teats.

Epidemiological investigation of bovine mastitis, status of infection, treatment pattern would provide useful management information to the producer, veterinarian and other mastitis control team members (Shitandi 2004). The overall prevalence of mastitis in the current study (70.1%) showed the presence of many subclinical cases (carriers), lack of herd health monitoring and empirical treatment of cases and deficiencies of the existing management. The finding of this study is in line with the previous reports by Biru (1989) and Bishi (1998), who respectively reported 67.4% and 69.8% prevalence of mastitis in dairy cows in different farms in and around Addis Ababa. On the other hand, it was found higher than the report of Biffa (1994) and Nessru et al. (1997), who reported a prevalence of 33% and 25%, respectively.

Over all quarter prevalence of 44.8% was recorded in the current study, which is comparable with the 37% reports of Nessru et al. (1997). But Biffa et al. (2005) and Almaw (2004) reported the higher quarter prevalence of 28.2% and 17.9%; respectively. The variability in the prevalence of bovine mastitis among the reports could be attributed to difference in management of the farms, breeds considered or technical know- how of the investigators. The quarter prevalence of clinical mastitis accounted for 10% where as the subclinical mastitis was 34.8%, which is in agreement with the reports on bovine clinical mastitis by Biffa et al. (2005)(12.2%) and Radostits (1994) (10%). On the other hand it was lower than the report of Workneh et al. (2002), who found a prevalence of 21.5% for clinical mastitis (Repi and Debre zeit). Subclinical 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 (Erskine 2001).

From the 149 CMT positive quarter milk samples, 15 (10%) were bacteriologically negative, which is inline with the report of Sori et al. (2005) who reported a prevalence of 9.82%. It was however; lower than the reports of Aregaw (1992) and Biffa (1994), who reported a proportion of 18%, 13.9% and 15%, respectively. The failure to isolate bacteria from the CMT-positive milk samples could be partly associated with spontaneous elimination of infection, low concentration of pathogen in milk, intermittent shedding of pathogen, intracellular location of pathogens and presence of inhibitory substance in milk (Radostitis 2000). It might also be due to some cases of delayed healing of infection from which organisms may have disappeared or reduced, while infiltration of leukocytes continued until complete healing (Sori et al., 2005).

In this study, most of the bacterial pathogens isolated from milk samples were Staphylococcus species, *S. aureus* (47.1%) being the predominant. In line with this, finding, Workneh et al. (2005), Nessru et al. (1997) and Vaarst and Envoldsen (1997) presented similar data on the primary role of S. aureus in bovine mastitis. The high prevalence of this organism may be associated with its frequent colonization of teats, its ability to exist intracelluarly and localize within micro abscesses in the udder and hence resistant to

Table 2: Summary of CMT score (n = 428) and culture result of mastitic cows

Forms of mastitis	CMT		Culture	
	No. positive	% positive	No. Cultured	% positive
Clinical	43	10.0	12	12 (100.0)
Sub clinical	149	34.8	149	134 (90.0)
Total	192	44.9	161	146 (90.7)

Table 3: Bacteria isolated from cows with clinical and subclinical mastitis

Organisms	Number of Isolates (%)		Total	
	Clinical Mastitis	Sub clinical Mastitis	Number	Proportion
S. aureus	6(3.9)	66(43.13)	72	47.05%
Coagulase negative Staphylococcus (CNS)	2(1.33)	44(28.75)	46	30.06%
Streptococcus spp	1(0.65)	10(6.53)	11	7.18%
Micrococcus spp	-	5(3.26)	5	3.26%
Klebsiella pneumonae	-	5(3.26)	5	3.26%
Escherichia coli	4(2.61)	3 (1.96)	7	4.57%
Corynebacterium spp	-	3 (1.96)	3	1.96%
Enterobacter aerogen	-	2 (1.33)	2	1.33%
Bacillus Spp	-	2 (1.33)	2	1.33%
Total	13	140	153	100%

antibiotic treatment (MacDonald 1997). The bacteria usually establish chronic, subclinical infections and are shed in the milk, which serves as a source of infection for other healthy cows during the milking process. Transmission among cows increase whenever there is lack of effective udder washing and drying, post-milking teat dip and drying, inter-cow hand-washing and disinfection, washing clothes and milking machine cups (Radostits 1994).

Streptococcal species identified in the present study (5.5%) was lower than reported by Zerihun (1996) (27%). This lower report of isolates in the current study might be partly associated with the widespread use of penicillin in the area, which is known to be effective to eradicate mastitis caused by Streptococcus species (Sori et al., 2005). In agreement with the finding of Workeneh et al. (2002), the present study also identified a low prevalence of Micrococcus species and *Klebsiela* (3.3%), *E.coli* (4.6%), *Corynebacterium* (2.0%), and *Bacillus species* (1.3%). Of all the isolates, contagious pathogens (particularly, S. aureus) showed greater frequency than others. The few mixed infection per animal and multiple infections per quarter (4.8%) observed in the study could be explained by poor milking and management practice. Cow with one quarter harboring multiple infections has been known to have greater reduction in production than a cow with a single pathogen (Sori et al., 2005). Moreover, the chance of developing drug resistance is higher especially at times of no drug sensitivity test prior treatment.

The increased prevalence of mastitis with age and parity reported in the present study is in agreement with the report of Biffa et al. (2005), who indicated an increase in the prevalence as lactation number and age increase. The age-multiparous-high prevalence relationship is explored to be due to all increase in teat patency and degree and frequency of previous exposure in multiparous old cows (Harmon 1994; Radostits 1994). The high prevalence of mastitis associated with udder/teat injuries (85.7%) reported in this study suggests unsatisfactory udder management and health. The presence of barbed wire fence and ticks around the udder were the customary causes of udder/teat injuries in the farms. Higher prevalence of mastitis reported in cows maintained in crackled concrete and muddy soil floor where manure and wet bedding were not frequently removed. This substantiates the importance of sanitation in the epidemiology of mastitis.

The high occurrence of mastitis-induced blind mammary quarters (7.2%), which has a paramount

Table 4: Association between some of the intrinsic factors with occurrence of mastitis in the study area

Risk	No. of quarter	No (%) positive for CM	No (%) positive for SCM (%)	Total	OR (95%CI)	P-value
Age:						
Old	63	4(6.4)	28	32	1	0.00
Young adult	197	15(7.6)	51(23.9)	66(33.5)	2.5(1.65, 3.86)	0.015
Adults	168	24(14.3)	70(41.7)	94(56.0)	2.0(1.15, 3.64)	
Parity:						
Few	202	16 (7.9)	52 (25.7)	68 (33.7)	1.0	0.00
Moderate	204	27 (13.2)	85 (41.7)	112 (54.9)	2.4 (1.6, 3.6)	0.058
Many	22	0	12 (54.5)	12 (54.5)	2.4 (0.97, 5.75)	

Table 5: Association between some of the selected extrinsic factors with the occurrence of mastitis in the study area

Risk Factors	Number examined	CMT results		OR (95% CI)	P-value
		No. positive	Percentage		
Udder Hygiene					
Washing/drying	342	144	42.1	1.0	0.434
Washing only	70	38	54.3	1.4 (0.5, 4.3)	0.033
Not at all	16	10	62.5	2.3 (0.8, 6.4)	
Floor type					
Good concrete	342	144	42.1	1.0	0.552
Bad concrete	48	25	52.0	1.4 (0.6, 3.3)	0.116
Muddy soil	38	23	60.5	2.1 (1.1, 4.2)	
Injury					
Absent	421	186	44.2	1	
Present	7	6	85.7	7.7 (0.9, 64.1)	0.041

and direct impact on the dairy economy at least by reducing the amount of milk produced, signifies the importance of the problem. Lack of screening tests and treatment of subclinical mastitis and inadequate follow up of clinical and chronic cases were believed to be the major reasons for the development of quarter's blindness. Though induced by persistent challenges of the mammary glands by microbial pathogens, it remains hidden and result in gradual destruction of the mammary tissues that ultimately causes non-functional quarters (Biffa 2005).

Conclusion

Inadequate hygienic condition of dairy environment, poor milking procedure, poor animal health service and lack of proper attention to health of the mammary gland were important for the high prevalence of mastitis factors in the study farms. Adequate housing with proper sanitation and regular screening for early detection and treatment, follow up of chronic case, culling of older cows with repeated attacks, avoiding consecutive milking and susceptibility testing of the mastitis pathogens before treatment are recommended to alleviate the problem.

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