Dairy Cattle Mastitis In and Around Sebeta, Ethiopia

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ABSTRACT

A total of 180 local and crossbred dairy cows were examined to determine the overall prevalence of mastitis in and around Sebeta using Californian Mastitis Test (CMT), which revealed the overall mastitis prevalence in the area as 52.78% (16.11% clinical and 36.67% sub-clinical cases). Of 134 bacterial isolates identified from CMT-positive samples sent to a laboratory for microbiological examination, the majority of isolates were *Staphylococcus aureus* (44.03%) followed by *Staphylococcus epidermidis* (14.93%) and *Micrococcus* species (6.72%), and lowest isolation rate was for *Escherichia coli* and *Pseudomonas aeruginosa* (0.75% each). Other species isolated include *Streptococcus agalactiae* (3.73%), *Streptococcus dysagalactiae* (4.48%), *Streptococcus uberis* and other species (2.99% each), *Corynebacterium bovis* and *Actinomyces pyogenes* (5.97% each), *Corynebacterium ulcerans* (2.24%), and *Bacillus* species (4.48%). Of 720 quarters examined, 27 (3.75%) had blind teats; 212 quarters (30.93%) showed evidence of infection of mastitis (from 693, the remaining number of functional quarters). The infection rate showed statistically significant difference between the lactating quarters and non-lactating quarters ($P < 0.01$). The infection rate also varied significantly ($P < 0.05$) between crossbred (56.29%) and local zebu (34.48%). The prevalence of mastitis also significantly differed between animals with teat lesion, teat end shape, and status of ventilation ($P < 0.01$), udder conformation ($P < 0.05$), type of management ($P < 0.01$), and frequency of barn cleaning ($P < 0.01$). In 53.13% of animals sampled and 31.68% of CMT-positive quarters, mixed infection per animal and per quarter was observed.

INTRODUCTION

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.[1] Most estimates have shown a 30% reduction in productivity per affected quarter and a 15% reduction in production per cow/lactation,[2,3] making the disease one of the most costly and serious problems affecting the dairy industry worldwide.[4] The disease generally involves interplay between management practice and infectious agents. Among various infectious

Table 1. Prevalence of Clinical and Sub-Clinical Mastitis in and around Sebeta.

Infection Status	Clinical Status		
	Clinical	Sub-Clinical	Total
Infected	29 (16.11%)	66 (36.67%)	95 (52.78%)
Non-infected	0	85	85
Total	29	151	180

Table 2. Prevalence of Mastitis in Different Breeds of Cows.

Breed	Mastitis Status			
	Infected	Non-Infected	Total	%
Local	10	19	29	34.48
Cross	85	66	151	56.29
Total	95	85	180	52.78

$\chi^2 = 4.65$, df = 1, $P < 0.05$.

Table 3. Mastitis Prevalence in Relation to Physiological State of Mammary Gland.

Infection	Physiologic State of the Mammary Gland		
	Lactating	Non-Lactating	Total
Mastitic	79 (48.77%)	16 (88.89%)	95 (52.78%)
Non-mastitic	83 (51.23%)	2 (11.11%)	85 (47.22%)
Total	162	18	180

$\chi^2 = 10.49$, df = 1, $P < 0.01$

agents, bacterial pathogens (the greatest share of these organisms) have been known to be widely distributed in the environment of dairy cows, constituting a threat to the mammary gland.[5]

In Ethiopia, even though the disease of mastitis has been known locally, it has not been studied systematically, making information available on the prevalence of disease and associated economic loss inadequate. The objective of the present study is to assess the overall prevalence of mastitis in local and crossbreed dairy cows, to isolate and identify major mastitis pathogens, and to assess risk factors of mastitis in and around Sebeta, which is found in the Oromiya Regional State of Ethiopia.

MATERIAL AND METHODS

Data were collected from 180 lactating and non-lactating indigenous and crossbreed cows, brought to the Alemgana wereda veterinary clinic for udder or teat treatment, another purpose, or via farm visits. The study was based on the questionnaire survey, physical and clinical examinations of clinical cases, screening tests using the Californian Mastitis test (CMT), aseptic meticulous sampling, and microbiological investigation (culture, stain, and biochemical tests on the pure isolates). The screening tests were prepared according to Schalm et al.[5] The microbiological techniques were prepared according to Carter[6] and Carter and Chengappa.[7] Animals were categorized on the basis of breed, physiological state (lactating and non-lactating), and presence or absence of risk factors (teat or udder lesions, herd size, the clinical state of mammary gland, udder conformation, milking routine, and management types).

Data Analysis

The prevalence of mastitis was calculated using percentage values, and the possible association of the disease with different risk factors was analyzed by using chi-squared (χ^2) test.

RESULTS

Of the total 180 animals utilized for the study purpose, the results showed prevalence of clinical and sub-clinical mastitis as 16.11% and 36.67%, respectively (Table 1).

Results of Intrinsic Risk Factors

As shown in Tables 2 and 3, a significantly higher infection rate was observed in crossbred cows and in non-lactating cows. The infection rate in cows with pendulous udder (77.78%) was significantly higher ($\chi^2 = 5.01$, degree of freedom [df] = 1, $P < 0.05$) than the non-pendulous udder (50.00%). The results also showed significantly higher infection rate ($\chi^2 = 11.36$, df = 1, $P < 0.01$) in cows with udder (teat) lesions (84%) than cows with normal udder or teats (47.74%). Cows with disk-shaped, inverted, pointed, and round-shaped teat ends had 88.46%, 61.54%, 54.17%, and

Table 4. Bacteria Species Isolated from Bovine Clinical and Sub-Clinical Mastitis

	Clinical Status					
	Clinical		Sub-Clinical		Pooled	
Species of Bacteria Identified	**N**	**%**	**N**	**%**	**N**	**%**
Staphylococcus aureus	16	34.04	43	49.43	59	44.03
Staphylococcus epidermidis	4	8.51	16	18.39	20	14.93
Micrococcus spp	5	10.64	4	4.60	9	6.72
Streptococcus agalactiae	3	6.38	2	2.30	5	3.73
Streptococcus dysagalactiae	2	4.26	4	4.60	6	4.48
Streptococcus uberis	3	6.38	1	1.15	4	2.99
Other *Streptococcus* spp	2	4.26	2	2.30	4	2.99
Actinomyces pyogenes	3	6.38	5	5.75	8	5.97
Corynebacterium bovis	4	8.51	4	4.60	8	5.97
Corynebacterium ulcerans	1	2.13	2	2.30	3	2.24
Bacillus spp	2	4.26	4	4.60	6	4.48
Escherichia coli	1	2.13	0	0	1	0.75
Pseudomonas aeruginosa	1	2.13	0	0	1	0.75
Total	47	100.0	87	100.0	134	100.0

40.86% rates of infection, respectively, and the difference was significant ($\chi^2 = 19.035$, df = 3, $P < 0.01$).

Result of Extrinsic Risk Factors

Out of 48 intensively, 107 semi-intensively, and 25 extensively managed cows studied, the highest infection rate was seen in intensively managed cows (66.67%) followed by the semi-intensive (52.34%), and the least for the extensively managed animals (28%). Infection rate of the mammary gland of these groups was significantly influenced ($\chi^2 = 9.87$, df = 1, $P < 0.01$). There was a higher prevalence of mastitis in poorly ventilated farms than in well-ventilated farms with infection rates of 87.5% and 49.39%, respectively; the result was significantly different ($P < 0.05$). The result showed no significant difference in farms that had drainage or in different group-sized herds, but there was a significant difference in farms that regularly cleaned their barn ($P < 0.01$).

Screening Test Result

Of 720 quarters examined, 20 animals (11.11%) had an overall incidence of 27 (3.75%) blind teats. From the remaining functional teats examined by CMT (693 quarters), 212 quarters (30.93%) showed evidence of clinical and sub-clinical mastitis of which 112 quarters samples were sent to a laboratory for microbiological examination; 101 (90.18%) were found bacteriologically positive. The infection was higher in left rear (34.3%) and right front (30.06%) quarters than in the remaining 2 quarters. The overall difference was not statistically significant.

Results of Bacteriological Examination

From the 95 CMT-positive cows tested, 64 cows (67.37%) were sampled for each of their positive quarters (25 animal from clinical and 39 animals from sub-clinical cases) from which a total of 134 bacterial isolates were identified (47 isolates from clinical and 87 isolates from sub-clinical mastitis cases).

The relative prevalence rates of various bacterial species isolated from the clinical and sub-clinical cases are shown in Table 4. Staphylococci were the major pathogens out of which *Staphylococcus aureus* contributed the major share (44.03%) accounting for 34.04% and 49.43% from clinical and sub-clinical mastitis cases, respectively. *Staphylococcus epidermidis* was isolated from clinical and sub-clinical mastitis at the rate of 8.51% and 18.39%, respectively.

Pseudomonas aeruginosa and *Escherichia coli* were isolated from only clinical (mastitis). The major streptococcal species were *Streptococcus agalactiae* and *Streptococcus uberis* from clinical cases while *Streptococcus dysagalactiae* was equally isolated from both cases. The result also showed single and mixed udder infections by different bacterial isolates (Table 5 and 6).

Table 5. Prevalence of Single and Mixed Udder Infections within Dairy Cows.

Bacterial Species	Number of Animals	Relative Prevalence (%)
Staphylococcus aureus	17	26.56
S. aureus and *Staphylococcus epidermidis*	5	7.81
S. aureus and *Streptococcus* spp	5	7.81
Staphylococcus and *Corynebacterium* spp	7	10.94
Streptococcus spp	3	4.69
Corynebacterium spp	3	4.69
Bacillus spp	2	3.13
Micrococcus spp	3	4.69
Corynebacterium and *Micrococcus* spp	1	1.56
Staphylococcus and others (≥2)	15	23.44
S. epidermidis and *Streptococcus* spp	2	3.13
Other non-*Streptococcus* spp	1	1.56
Total	64	100.0

Table 6. Multiplicity of Infection per Individual Quarters from Both Clinical and Sub-Clinical Bovine Mastitis.

No.	Bacterial Isolates	Quarters	Relative Prevalence
1	*Micrococcus* spp and *Actinomyces pyogenes*	1	3.13
2	*Corynebacterium bovis* and *Staphylococcus aureus*	3	9.38
3	*Streptococcus agalactiae* and *S. aureus*	1	3.13
4	*S. aureus* and *Streptococcus dysagalactiae*	2	6.25
5	*S. aureus* and *Staphylococcus epidermidis*	2	6.25
6	*S. aureus* and *A. pyogenes*	5	15.63
7	*S. aureus, S. epidermidis,* and *Corynebacterium ulcerans*	1	3.13
8	*A. pyogenes* and *S. epidermidis*	1	3.13
9	*C. ulcerans* and *S. aureus*	1	3.13
10	*Micrococcus* spp and *S. epidermidis*	2	6.25
11	*S. aureus* and *Streptococcus uberis*	1	3.13
12	*S. epidermidis* and *S. dysagalactiae*	1	3.13
13	*S. epidermidis* and *S. agalactiae*	1	3.13
14	*S. agalactiae* and other *Streptococcus* spp	2	6.25
15	*S. aureus* and *Micrococcus* spp	1	3.13
16	*S. aureus* and *Bacillus* spp	2	6.25
17	*S. epidermidis* and *Pseudomonas aeruginosa*	1	3.13
18	*S. uberis* and *C. bovis*	1	3.13
19	*S. dysagalactiae* and *C. bovis*	1	3.13
20	*S. agalactiae* and *Micrococcus* spp	1	3.13
21	*S. dysagalactiae* and *Micrococcus* spp	1	3.13
	Total	32	100.0

Of the total isolates, contagious pathogens (*S. aureus*, *S. agalactiae*, and *S. dysagalactiae*), environmental pathogens (*E. coli*, *S. uberis*, and others), and dry cow pathogens (*Actinomyces pyogene*, *Micrococcus* spp.) account for 51.47%, 6.62%, and 1.47%, respectively. The rest (40.44%) corresponds to other unclassified pathogens.

DISCUSSION

This study showed the overall prevalence of mastitis in local and crossbreed cows in and around Sebeta to be 52.78%, which is in agreement with the reports on bovine mastitis reported by Tolossa[8] (53.5%) and Haile[9] (53.35%). On the other hand, the report of Biffa[10] (33.0%) was lower than the present findings, and the reports of Biru[11] (63.4%), Tolla[12] (61.11%), and Zerihun[13] (68.1%) were higher than the present findings. The variability in the prevalence of bovine mastitis between reports could be attributed to difference in management of the farms, breeds considered, or technical know-how of the investigators.

In this study the clinical mastitis accounted for 16.11% where as the sub-clinical mastitis was 36.67% of the share. The reports of Biffa[10] (15.1%) and Radostits and Blood[3] (10%) on clinical bovine mastitis was the same and lower than the present result. 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.[14]

Higher-yielding cows have been found more susceptible to mastitis[3] owing to position of teat and udder and anatomy of teat canal, making them prone to injury,[3] and due to less efficacy of phagoacytic cells in higher yielding cows[5] associated to dilution. In line with this, it was found in this study that the prevalence of mastitis in crossbred cows was statistically higher than that of local cattle ($P < 0.05$).

The rate of infection showed strong significant variation ($P < 0.01$) between lactating and non-locating cows, and between quarters that were lactating, lactating but approached drying, or dry. The dry period secretion has been reported to inhibit phagocytic action of neutrophils in the udder and, during dry period, the capacity of the quarters to provide phagocytic and bactericidal activities has been known to diminish, leading to high infection rate.[3,15] The higher prevalence of mastitis in cows with pendulous udder and those with teat and udder lesions in this study could be explained by the fact that pendulous udder exposes teat and udder to injury, and the pathogens may easily adhere to the latter, getting access to the gland tissue.[5,16]

The highest prevalence of mastitis in cows with disk-shaped, inverted, and pointed teat ends in this study may be explained by the presence of wider streak canals, which have been shown to allow more pathogen penetration[17] in former cases and by the fact that pointed teat ends have a narrower streak canal, exposing the teat to more milking periods and irritation.[18] The more prevalence of infection in poorly ventilated barns (87.5%), in barns with beddings (50.3%), and in barns without drainage (60.86%) indicates that these risk factors contribute to mastitis in the herd, and the quarter prevalence of 30.59% obtained by this study indicates the economic significance of the disease.[2,3]

The relative high prevalence of mastitis in left rear quarters (34.3%) and right front quarter (30.06%) in this study agrees with the finding of others.[4,19] This may be due to greater production capacity of hindquarters,[5] the likelihood of fecal and environmental contamination of hindquarters, and ease of first grasping by milker's hand in case of right front quarters.

This result showed *S. aureus* and *S. epidermidis* as the major pathogens of mastitis in the area that might be due to lack of effective udder washing and drying, post-milking teat dip and drying, inter-cow hand-washing, and disinfection in the milking routine of the area. Contamination of milkers' hands, wash clothes, and milking

machine cups by milk from infected quarter has been reported to quickly lead to spread of mastitis.[3]

The cumulative incidence of streptococcal mastitis during this survey (14.18%) was lower than the amount reported for the same species by Kingwill et al.[20] (80.95%), Tolossa[8] (53.55%), and Zerihun[13] (27%) in dairy cows. The lower isolation rate in this study might be associated with the widespread use of penicillin in the area for treatment of mastitis. It has been recognized that mastitis caused by *Streptococcus* species is susceptible to eradication via use of penicillin.[3,5]

The relative higher prevalence of *Corynebacterium* mastitis in this study (14.18%) compared with reports of Biru[11] (4.2%), Biffa[10] (4.55%), Tolla[12] (6.66%), and Zerihun[13] (1.9%) is consistent with former reports by Schalm et al.[5] and Radostis and Blood[3] that higher incidence of *Corynebacterium bovis* is associated with lack of post-milking teat dip and that *Actinomyces pyogenes* follows teat or udder injury as observed in the study area.

The low prevalence of *Pseudomonas* species (0.75%) in this study could be attributed to the intermittent shading nature of this organism from the udder to milk.[3] The low prevalence of *E. coli* could be due to higher prevalence of other pathogens.

Of all the isolates, contagious pathogens showed greater frequency than others (*S. aureus* was the most common). In 53.13% of the cows and in 32 quarters sampled, mixed infection per animal and multiple infections per quarter were found. This could be explained by poor milking and management practices and hired milkers who milk in more than one farm. Such mixed infection per cow per quarter indicates the economic significance of the disease in the area. A cow with 1 quarter harboring multiple infections has been known to have greater reduction in production than a cow with a single pathogen.[21]

From the 112 quarter samples sent to a laboratory for microbiological investigation, 11 (9.82%) were bacteriologically negative, which is lower than the observation by Pearson and Mackie,[22] Aregaw,[23] and Biffa[10] who reported proportions of 18%, 13.86%, and 15%, respectively. The failure to isolate the bacteria from the CMT-positive samples could be attributed to bactericidal properties of the inflammatory udder secretions, which have been known to destroy the infecting bacteria leaving milk with higher leukocyte counts.[24] It might also be due to some cases of delayed healing of infections from which organisms may have disappeared or been reduced, while the infiltration of leukocytes continued until complete healing.[5] The present study revealed the seriousness of mastitis in the area and significance of its contributors in the area. A practical mastitis control strategy in the herd and national approach is needed.

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