



Screening Test for Prevalence of Subclinical Mastitis in Small Dairy Animal Holders in and Around Sebeta Town

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Abstract

Across sectional study was conducted from August to September 2021 to determine the prevalence of mastitis in locating dairy cows that are found in and around selected kebeles of Sebeta town. Age, breed, lactation period, calving interval and management of the animals were taken into consideration as risk factors. A total of 100 lactating cows (16 local, 20 cross and 64 exotic) were examined for mastitis using California Mastitis Test (CMT) and 63% of individual cows were screened to be test positive. An overall prevalence of (55.96%) was also recorded at quarter level. Higher rate of infection was determined in adult (70.18%) than in old (59.09%) and young (47.62%) cows, but statistical difference ($P=0.852$) in the prevalence of disease was not observed between those other age group. Statistically significant difference ($P=0.031$) in prevalence of mastitis was observed between cross (59.39%) and local breeds (81.25%) and exotic (60.00%). Cows in early lactation period (64.39%) were highly prevalent with mastitis than cows in late (62.50) and early lactation period (33.33%) with ($P=0.0321$). Besides, prevalence of 65.79%, and equal prevalence of (61.29%) was respectively determined in extensive, intensive and semi intensive management systems. In conclusion, the present study showed that mastitis is common in selected study areas and appropriate control strategies have to be designed to reduce the effect of the disease.

Keywords: California mastitis test; Mastitis; Prevalence; Quarter level

Introduction

In Ethiopia livestock represent a major national resource and form an integral part of the agricultural production system cows represent the largest proportion of cattle population of Ethiopia in which 55.5% of the total cattle heads for the private holdings are milking cows. However, compared to other country in Africa, Ethiopia consumes less dairy cattle products. Bovine mastitis is a large scale infectious disease

with significant impact on the economy of milk production [1]. Besides health disorders of the mammary gland, mastitis can also cause significant losses in milk yield, alterations in its quality (impaired nutritive and technological properties of milk), fertility disorders and even systemic diseases [2-5].

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 [6]. Moreover, due to its latent form, heavy financial losses and great nutritional and technological impacts can be resulted. Because valuable components of the carbohydrate, proteins, fats, vitamins and minerals are decreased. Milk like lactose, fat and casein are decreased while the increase in human population, accessibility to undesirable components like those that ions and enzymes are increased and making the milk unfit for processing technology [7,8]. Mastitis is the most important and expensive disease of dairy industry. It also results in severe economic losses from treatment cost, increased labor milk withheld following treatment and premature culling [9]. It is recognized that if this disease is diagnosed in early stages. The greater portion of this loss can be avoided. This disease is characterized by physical, chemical and bacteriological changes in the milk and pathological changes in the tissue of the udder. The most great changes occurred in the milk when it is infected by mastitis in include discoloration. Presence of clots and large number of leukocytes, the bacterial contamination of milk from affected cows render it unfit for human consumption, as it can provide a mechanism of spread of disease like tuberculosis, store throat, brucellosis and leptospirosis and has zoonotic importance.

Two distinct patterns in the epidemiology of mastitis can be categorized. The first pattern is a contagious disease pattern where transfer of microorganisms from animal to animal is essential to propagate the disease. These major microorganisms are *Streptococcus agalactiae* and *Staphylococcus aureus*. Contagious disease remains endemic when the number of susceptible individuals infected by an infected individual is appreciably larger. The second pattern is opportunistic disease pattern. Microorganisms, host factors and environmental factors put an animal at risk. The most important microorganisms involved are the coliforms (*E.coli* and *Klebsiella* species) and the environmental streptococci [10].

The etiologic agent of mastitis can be categorized in to two major ways based on their modes of transmission. Those can be contagious and environmental pathogens. The primary reservoir of contagious pathogens is an infected udder whereas a contaminated environment is the primary reservoir of pathogens causing environmental mastitis. *Streptococcus agalactiae*, *Staphylococcus aureus* sub species aureus and mycoplasma species are considered as typical contagious pathogens. *Streptococcus dysgalactiae* has been most commonly described as a contagious pathogen but it can also behave as an environmental pathogen. Coagulase Negative *Staphylococcus* (CNS) is the prevalent bacterial pathogen in the udder infections. These pathogens spread from infected to clean udders during the milking process through contaminated milkers hand and by flies. Transmission of the environmental pathogens occurs between milking process. Some infections are usually associated with unsanitary environment, while the other are found in sawdust that contains bark soil.

The pathogenesis of mastitis can occur through the invasion from outside or through the internal invasion of the mammary gland. In

subclinical mastitis, there is no visible sign observed and its pathogenic effect is not easily understood unless CMT screening is adopted. But in clinical mastitis the infection leads to desquamation of connective tissue of the udder as result, thickening and exudation occur then hot, painful swelling of udder will be followed. In chronic form of the disease part of the udder tissue, get fibrosed [11]. There are different ways by which dairy mastitis can be diagnosed. Somatic Cell Count (SCC). California Mastitis Test (CMT), enzymatic method and conductometry have been mainly used for the diagnosis of mastitis in dairys cattle [12]. According to Radostits, the diagnosis of bovine mastitis is performed by clinical examination (inspection and palpation) for clinical forms of mastitis and screening (CMT) test, bacterial isolation; confirmatory diagnosis for subclinical forms of mastitis will be used.

The most important way to continuously produce milk of good quality is to keep the mastitis situation in the herd under tight control. It is one of the most complex diseases of dairy cows that generally involve interplay between management practices and infectious agents, having various causes and degrees of intensity. It is highly prevalent problem in dairy cattle and is one of the most important threats affecting the world's dairy industry [13]. Therefore the main objective of this study was to screen out the bacterial pathogens that cause sub clinical mastitis in dairy cows in the study areas of Sebeta town; central highland of Ethiopia known by market oriented dairy cows.

Materials and Methods

Study area

The study was conducted in and around Sebeta which is located on the southwestern cost of Addis Ababa located 08°9'20" North and 38°6'20" East. The mean annual rainfall and temperature of the town are 1073 milliliters and 17.40°C, respectively. The altitude ranges from 2356-2405 m above sea level. The aggregation was purposely selected mainly due to presence of many commercial and semi commercial farms with mixed crop and livestock farming system is the mode of agriculture in the districts.

Study animal population

The study animals were dairy cows of both local and cross breeds which were selected from small dairy farmer holders purposively used for milk consumption. They are managed under extensive, intensive and semi-intensive management system. According to the owners response almost most of the cows are managed under extensive management system and they feed by grazing on natural pasture but intermittently they are given crops such as maize, sorghum and other depending on the availability of an extra feeds. Others are managed under semi intensive when delivery time arrives and at early stage of lactation. Data regarding to the different risk factors such as age, lactation stage, breed, calving interval and management system were assessed.

Study design

A cross sectional study design was conducted in order to design the screening test to determine the prevalence of bovine mastitis in lactating dairy cows using California mastitis test. Then simple random sampling was employed to select individual study cows. The study involved physical examination of sampled cows and collection

of milk samples following standard procedures. The samples from each functional udder of cows were screened for sub-clinical mastitis using the California Mastitis Test.

Sampling method and sample size determination

The purposive sampling method was the solely method which was used while collecting samples. The sample size was determined based on the formula given by Thrusfield considering, 5% absolute precision, 95% confidence interval and from previos studies around Holeta town, with an expected prevalence of 71% [14]. Therefore, the total sample size was 316 based on the given formula below but in order to increase the precession a total of 347 lactating cows were used.

$$n=1.962 * Pexp * (1-Pexp) d^2$$

Where, n=required sample size

Pexp=expected prevalence

d=desired absolute precision

z=confidence internal

Based on this 100 individual cows were examined to screen out the bovine with sub clinical mastitis using CMT reagents provided from NADHIC regional laboratory.

Physical examination of the under and milk

The udder of each cow was physically inspected for its cleanness, swollen and redness which is associated to mastitis. Every sampled milk was inspected physically as that of udder for its color and consistency.

Milk sample collection and transportation

During milk sample collection, cows were restrained in standing position and sampling began with teat cleaning by scrubbing thoroughly using cotton balls moistened with 70% alcohol. Milk samples were collected first from the closest teats followed by those at the far side of the udder by maintaining universal bottle at approximately 45° angles following the methods described by Quinn, et al., strict aseptic procedures was adopted when collecting milk samples in order to prevent contamination with microorganisms present on the body of animal and from the environment. Milk samples were collected according to National Mastitis Council (NMC). The teat of the udder was first disinfected by (70%) alcohol and dried. To reduce contamination of the teat ends during sample collection, the near teats was sampled first followed by the far once. Approximately 10 ml of milk was collected in to a sterile test tube after discarding the first three milking stream. After collection of milk sample, all samples were clearly labeled with the appropriate identification criteria using permanent marker on the test tube. Then samples was placed in racks for ease of handling and transported in an ice box to the National Animal Health Diagnostic Investigation Center (NADHIC) laboratory for CMT screening test.

CMT screening strategies

The California Mastitis Test (CMT) was carried out according to the method described by Quinn, et al. A squirt of milk, about 2 ml from each quarter was placed in each of four shallow cups in the CMT paddle. An equal amount of reagent was added to each cup. A gentle

circular motion was applied to the mixtures, in a horizontal plane for 15 seconds. The milk reagent mixture was swirled in a circular motion with presence of gel or slime being recorded for each quarter. Measurable response in the paddle and reaction score results were given based on the degree of precipitation or gel formation [15]. The results were classified as either negative or positive depending on the intensity of the reaction which is showed by gel formation samples

with a CMT score of 0 or Trace were considered as negative while those with CMT scores of 1, 2 and 3 were considered as positive. The degree of intensity of reaction with the CMT reagent was ranked and recorded as negative if there is no any reaction, weak positive, distinctive positive if the reaction shows thickening and viscous cluster; strong positive if the thick gel consistency was observed (Table 1).

Reading aspects	Score value	CMT score	Interpretation
Normal consistency	0	Negative	Absent
Light gel disappearing; After stirring	1	Weak positive	Infection by minor pathogens
Thick gel consistency	2	Strong positive	Subclinical mastitis which in progress in to clinical mastitis

Table 1: Scoring of California mastitis test.

Data management and analysis

The data collected during the study periods were entered into MS. Excel 2007 spread sheet and filtered and analyzed by using a soft ware of SPPSS V16. Descriptive statistics was used to estimate the frequencies. Logistic regression was used to see the association between different risk factors such as age, lactation stage, breed and calving intervals. $P < 0.05$ were considered as significant.

Results

Prevalence of bovine mastitis

An overall prevalence of 63.00% bovine mastitis was recorded in this present study conducted on total of 100 lactating dairy individual

cows and 386 each and every individual teat (Table 2). Among this, 62% tested using hind right udder 56.84% hind left udder, 58.33% on front right udder and 46.32% using front left were respectively screened to be positive due to clinical or subclinical mastitis. A total of 386 teats were examined, for detection mastitis at quarter level. The overall prevalence obtained was 55.96% after each and every teats were examined as indicated at Table 3. Mastitis prevalence was found to be 81.25 % in local, 59.38 in exotic and 60.00% cross breeds. Statistical analysis showed the existence of significant difference ($P < 0.05$) between the two breeds as indicated at Table 2.

Quarters	Number of examined teats	CMT positive	Prevalence
Hind right quarters	100	62	62.00%
Hind Left quarters	95	54	56.84%
Front rights	96	56	58.33%
Front left	95	44	46.32%
Total of teats	386	216	55.96%

Table 2: Prevalence of bovine mastitis at quarter level.

The pathogens causing the bovine mastitis in association with different risk factors such breeds, age, lactation period, calving interval and management system was screen out and the result shows the prevalence of bovine mastitis studied using screening test of CMT was found to be 81.25 % in local, 59.38% in exotic and 60.00 % cross

breeds. The result shows that there is significantly associated with breeds ($P < 0.05$), lactation period, and management and but no variation was recorded as indicated in at table 3 in age group of adult and young dairy cows with except there is a significant association of microbial pathogens to cause the subclinical bovine mastitis as indicated in a Table 3.

Risk factors	Category	No of cows examined	No of cows (+ve) to CMT	Prevalence (%)	P-value
Breed	Local	16	13	81.25%	0.031
	Cross	20	12	60.00%	0.034
	Exotic	64	38	59.38%	0.028

Age	Old	22	13	59.09%	0.041
	Young	21	10	47.62%	0.06
	Adult	57	40	70.18%	0.057
Lactation period	Early	3	1	33.33%	0.034
	Mid	24	15	64.39%	0.0321
	Late	73	47	62.50%	0.034
Calving interval	Moderate	72	18	72.22%	0.74
	Few	15	15	54.54%	0.06
	Many	18	15	36.36%	0.079
Management	Extensive	38	25	65.79%	0.026
	Intensive	31	19	61.29%	0.028
	Semi I	31	19	61.29%	0.021

Table 3: Analysis result of risk factors for the occurrence of bovine mastitis at selected dairy cows of small holder farmers.

According to the data shows, calving intervals is not significantly associated with the causative pathogens of bovine mastitis which at any calving intervals if there is appropriate control measures, there is no way to be diseased by bovine mastitis.

Discussion

The overall prevalence of bovine mastitis were studied within different risk groups using breeds, age, lactation period, calving interval and managements. Total prevalence recorded both in local and cross breeds were 59.38% and 60.00%. Which were in close agreement with previous findings of other authors in different regions of Ethiopia like: 36.9%, 38.6%, 38.2%, 36.7% and dire dawa in eastern Ethiopia, in chaffe valley and respectively [16]. However, the current prevalence in the study area is less than the previous findings of different authors in different regions of Ethiopia like: 44.6%, 45.9%, 46.7%, 52.8%, 61.1%, which was reported by in soddo in Adama in Sebeke, in south wollo [17,18].

The prevalence of bovine mastitis in the present study is greater than the previous findings of different authors like: 34.9%, 33%, 25%, and 28.2% which was forwarded and in Bahir Dar respectively, on quarter level [19,20]. But it has some similarities with the findings of Almag. When compared to other teats the hind teats, hind right and front teats; front right teats are much more affected. Their prevalence were 62.00% and 58.38% respectively, but in both front teats, front right and front left are affected next to the former two teats and their prevalence were 16.1% and 12.4% respectively. As compared to the others, the left hind quarters were affected with the highest infection rate than right front. The right hind quarters were the second with an infection rate. This might be due to the high production capacity of the hind quarters and the high chance of getting fecal and environmental contamination [21,22].

Difference in breed can also play a crucial role in the prevalence of mastitis. The types of breed that was used for the study purpose was exotic, local and cross breeds of animal which are collected from small dairy holders from selected district of Sebeta town. Prevalence

of mastitis was significantly ($P < 0.05$) associated with the breeds and management system. This agrees with the findings of Demelash.

Age is a detrimental factor in the distribution of the mastitis. Therefore it was taken into consideration to measure the prevalence of mastitis and was measured for different age groups of lactating cows. The prevalence was found to be much higher in the old and adult age group than the young age group but there is no statistical significant difference between the young and adult age groups; whereas significantly old aged groups of dairy animals are much suspected for pathogens causing bovine mastitis. Management system was also considered as risk factors for prevalence of mastitis and categorized as extensive, intensive and semi intensive management system. The prevalence of the disease occurrence was nearly equal in the second two management system and greater in first one. But statistically, there is no difference among the management systems.

Conclusion

The present study showed that an overall prevalence of 55.96 % bovine mastitis was recorded by using screening test of California mastitis test at quarter level. Among the different risk factors were considered during this study; whereas breeds, lactation periods and management systems are statistically significant difference ($P < 0.05$). Based on the above conclusion following recommendations are forwarded. The only old aged dairy animals were found to be significantly associated with the occurrence of bovine mastitis.

- Awareness creation should be given to the farmers on the impacts of bovine mastitis.
- Attention should be given to sub-clinical mastitis since it does not show any obvious signs to the farmer or to the owner of animals, its' impact on lactating dairy cows is more exaggerated than clinical mastitis.
- As there is no sufficient information about bovine mastitis in the study area, further study should be conducted.

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