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Research Article

Prevalence and Incidence of Bovine Mastitis in Dairy Farm of Haramaya University, Eastern Ethiopia

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Abstract

Mastitis is an inflammation of parenchyma of mammary glands resulted in physical or pathological changes in glandular tissues. In this study both cross sectional and follow up study was conducted on 56 study animals to determine prevalence and incidence of bovine mastitis, determination of risk factors for occurrence of mastitis and bacterial isolation from March to October, 2021. Cohort study design was conducted to determine the incidence of subclinical bovine mastitis on 32 negative animals to mastitis after first screening. The prevalence of clinical and subclinical mastitis was 12.5% and 30.4% respectively out of which 7.1% cows had blind teat. Cows having >5 calving, late stages of lactation, >6 years age and cow giving milk yield to >15 liters had higher prevalence as compared to other relevant groups. The incidence rate of 2.04 and 1.57 cases per 100 cows per week were recorded in cow-wise and quarter-wise respectively for subclinical mastitis. From observed risk factor associated with incidence of subclinical mastitis, milk yield had statistically significance association for cow-wise incidence. Milk yield, lactation stage and breed had again association with quarter-wise incidence. From bacterial isolates *Staphylococcus* species 54(48.21%), and *Escherichiacoli* 22(19.64%) were the most two species isolated in the present study. The present high prevalence and incidence of mastitis in the study farm need regular screening of subclinical mastitis for early detection and management, and proper sanitary conditions of cow's environments. Furthermore, antibiotic resistant test and seasonal incidence rate determinations were forwarded as research gap.

Keywords: Bacterial isolation; Dairy farm; Haramaya university; Incidence rate; Mastitis; Risk factors

Introduction

Bovine mastitis remains one of the most critical diseases of dairy cows globally [17]. It is multi-etiological and complex disease, which is defined as inflammation of parenchyma of mammary glands, characterized by physical, chemical and usually bacteriological changes in milk, and pathological changes in glandular tissues [11]. Mastitis can be occur as clinical or subclinical forms based on inflammation of mammary gland with grossly visible changes on the udder and milk abnormalities [24,30].

This complex disease had relation with different factors including individual cow-level risk factors such as parity, stage of lactation, breed, age, udder hygiene and dry cow therapy [23,29].

Majority of microorganisms that are responsible for mastitis are of bacterial origin including *Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis*, *Mycoplasma* species, *Streptococcus uberis*, coliforms (*Escherichiacoli*, *Klebsiella* species, and *Enterobacter aerogenes*), *Serratia*, *Pseudomonas*, *Proteus* species, environmental *Streptococci*, and *Enterobacter* species [31].

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Subclinical form of mastitis has received little attention in Ethiopia, [13]. But, the disease causes annual production losses of 22.3% in cross breed cows and 2.24% in local breed lactating cows in the production cycle in one year [3]. In Ethiopia more prevalence study had conducted in different parts of the country including the work of [2,3,6,16,20,26] and many others. However, incidence of mastitis has not been studied like that of prevalence, so that it needs more attention and investigations for early management of the disease.

The prevalence of both clinical and subclinical mastitis was also scientifically not well documented in the present targeted dairy farm. Therefore the above realities and problems initiated this work with the objectives of estimating prevalence and incidence of bovine mastitis, determine risk factors responsible for occurrence of the disease and isolate bacteria responsible for the disease in Dairy farm of Haramaya University, Eastern Ethiopia.

Materials and Methods

Study Area

The study was conducted at Haramaya University Dairy Farm which is located in the East Hararghe Zone of Oromia Regional State, Ethiopia. The area was at the distance of about 508km east of Addis Ababa, the capital city of Ethiopia. The area is located at altitude of 1600-2100m above sea level, 410 59' 58" latitude and 090 10' 24" longitudes. Study area has 18°C mean annual temperature and 65% relative humidity and receives 900mm annual rainfall with a bimodal distribution pattern, peaking in mid-April and mid-August. Main pasture production is expected after the short rain season, continuing until the end of the long wet season [19].

Study Population and Husbandry Practices

The farm has 148 dairy cattle that are kept under intensive production system by Haramaya University. The target population for this study was lactating dairy cattle of Holstein cross breed and Jersey cross breed kept under the same Management (intensive farming system with concrete bedding, good ventilation and provision of supplementary diets in addition to the agricultural by-products). The cows were milked twice a day using common milking machine. The cow attributes include age in year (<5, 5-6, and >6), breed (Holstein cross and Jersey), parity (1, 2, 3, 4 >4) and stage of lactation in months (early 1–3, mid 4–6, and late ≥7 months), and milk yield (<5, 5-10, 10-15, >15liters).

Sample Size and Sampling Method

A total of 56 lactating cows selected purposively from the target population were first tested for prevalence of mastitis using physical examination and CMT. Then those 32 cows negative from both clinical and subclinical mastitis at the first test were followed and checked in a week interval during the study period for incidence of subclinical mastitis. For this study milk samples were collected from cow positive for mastitis by using sterile test tube and transported to laboratory for identification of the major bacterial pathogens causing mastitis. Before sampling, the udder and teats were washed with tap water and dried, and then the teat ends was vigorously swabbed with gauze cloth soaked in 70% ethyl alcohol and a few streams of milk from the teat (strict foremilk) were discard. For the bacteriological examination, milk sample was collected from each infected teat of individual cows and labeled and transported by icebox to Haramaya University Veterinary Microbiology Laboratory, and stored at 4°C for a maximum of 24 hours until inoculation on a standard prepared bacteriological media.

Study Design

A cross sectional study was conducted from March, 2021 to October, 2021 and all the study animals were tested at the beginning of the study period for prevalence of clinical and subclinical mastitis by physical examination of the udder for any abnormalities and CMT respectively. Then a Cohort study design was conducted to determine the incidence of sub-clinical bovine mastitis. The farm was visited weekly and cows were tested by CMT at quarter level and result recorded throughout the study period. Risk factors were studied in association with both prevalence and incidence, while bacterial causative agents were isolated from mastitis quarters during incidence study following standard procedures [22].

California Mastitis Test

Every week lactating dairy cows, which were negative for both clinical and subclinical mastitis at the first visit, were tested with CMT during the study period. Milk secretion from those negative cows were checked by California Mastitis Test by putting CMT reagent in the 4 cups of the CMT paddle in which milk from the respective quarters of the cow was added with equal amount of CMT reagent and gently mixed by rotating the paddle for about 30 seconds. The test result interpretation is based on the thickness of the gel formed. A cow that had one or more positive quarters to CMT was considered positive for subclinical mastitis.

Bacterial Isolation and Identification

For bacteriological examination, refrigerated milk samples were warm at room temperature and mixed by shaking in order to disperse bacteria and fat, and then stand to disperse foam before just inoculation. A loopful of milk sample was streaked on blood agar base enriched with 5% defibrinated sheep blood. The inoculated blood agar plates were incubated aerobically at 37°C and checked for growth after 24 hours, 48 hours, and 72 hours. The plates were examined for growth, morphological features, such as colony size, shape, and color, and hemolytic characteristics. Suspected colonies were cultured on nutrient agar plate from which colony was taken for further investigation.

Bacterial isolation and identifications were based on standard procedures set in Quinn et al. (2004) [22]. Gram staining was done for the identification of Gram-positive and Gram-negative bacteria, and colony morphology (coccus or rods). Tests such as catalase test, oxidase test, motility test, oxidation fermentation test, and growth or absence of bacteria on Mac-Conkey agar, were done using standard procedures. Other biochemical tests like IMViC (Indole test, methyl red, Voges-Proskauer) tests were done for further identification.

Data Analysis

Data generated from continuous observation and laboratory investigations was recorded and coded using Microsoft Excel 2010 spreadsheet and analyzed using STATA version 16.0 for Windows (StataCorp. College Station, Texas 77845 USA). The prevalence was calculated as the number of cases during the study period divided by the total number of animals examined during the study and the incidence was calculated as the number of new cases during the study period divided by the total

number of animals at risk in given time. The association of risk factors (breed, parity, age, stage of lactation, and milk yield) on occurrence of mastitis was computed by Pearson's Chi-square ($\chi 2$) and $P<0.05$ used as statistically significant value.

Results

In the present study from 56 total lactating dairy cattle examined at the beginning of the study, 24(42.9%) dairy cows became positive for both clinical and subclinical mastitis. From 24 positive cows 7(12.5%) were affected by clinical mastitis and 17(30.4%) cows were affected by subclinical mastitis (Table 1).

Table 1: The prevalence of clinical and subclinical mastitis at HU Dairy farm.

Form of mastitis	No examined	No. Positive	Prevalence (%)
Clinical	56	7	12.5
Sub clinical	56	17	30.4
Blind teat	56	4	7.1

The result of the present study revealed that, except breed, all risk factors have a significant association with the prevalence of clinical mastitis. The highest prevalence was recorded in cows having >5 calve (5.36%), late lactation stage (12.5%), >6 years aged (8.93%), and cow giving milk yield >15liters (12.5%) when compared to the others. Cows at late lactation stages had higher prevalence (21.43%) than other stage, where as prevalence of (17.86%) was recorded in cows giving milk yield to >15liters/day than less milk production with statistically significant difference (Table 2). However, only lactation stage and milk yield were found to be having a significant effect on the prevalence of subclinical mastitis.

Out of 32 lactating cows followed for onset of subclinical mastitis, 28/32(87.5%) cows and 112/128(87.5%) quarters had mastitis in four months period of follow-up as new cases. The incidence rate of 2.04 cases per 100 cows per week and the incidence rate of 1.57 quarters per 100 quarters per week were recorded for cow-wise and quarter-wise respectively during four months period.

Only milk yield was found to be having a significant effect on the Incidence of subclinical mastitis ($P<0.05$) as indicated in (Table 3). Higher Incidence rate (4.76) was recorded in cows giving milk yield to >15liters as compared to other group of milk production. In quarter level breed, Lactation stage and milk yield were found to be having a significant effect on the incidence of subclinical mastitis. Jersey cross breed cows had a higher incidence rate (3.12) than Holstein crossbreed that had an incidence rate of 1.38. Cows at mid lactation stages had incidence rates of 2.84 with significance difference than others. Higher incidence rate (3.57) was recorded in cows giving milk yield to >15liters as compared to other groups (Table 3).

From total of 128 quarter examined, 112 teat quarters were CMT positive and milk samples were collected at quarter level for bacterial identification. A typical colony was focused for subculture and further process in bacterial species identification. Accordingly, all collected samples were culture positive with highest isolation rate of *Staphylococcus* species 54(48.21%), *Escherichia coli* 22(19.64%) and *Streptococcus* species 14(12.5) respectively. With respect to species prevalence *Staphylococcus aureus* 36(32.14%) and with least isolation rate of *Streptococcus* uberis and *Streptococcus feacalis* at isolation rate of 3(2.68%) each were reported (Table 4).

Table 2: Association b/n risk factors and Prevalence of Bovine clinical and subclinical mastitis in the study farm (N=56).

	Clinical Subclinical							
Animal Category	**No Positive**	**Prevalence (%)**	**χ^2**	**P-value**	**No Positive**	**Prevalence (%)**	**χ^2**	**P-value**
Breed								
Holstein cross	6	10.71	0.0233	0.879	17	30.36	3.487	0.062
Jersey cross	1	1.79			0	0		
Parity								
1 Ccalve	0	0	23.547	0.001	1	1.79	2.921	0.571
2 Calve	1	1.79			7	12.5		
3 Calve	2	3.57			8	14.29		
4 Calve	1	1.79			1	1.79		
>5 Calve	3	5.36			0	0		
Lactation								
Early	0	0	9.92	0.007	2	3.57	6.988	0.03
Mid	0	0			3	5.36		
Late	7	12.5			12	21.43		
Age								
<5 years	0	0	18.247	0.001	0	0	4.275	0.118
5-6 years	2	3.57			16	28.57		
>6 years	5	8.93			1	1.79		
Milk yield								
<5 liters	0	0	15.578	0.001	0	0	14.97	0.002
5-10 liters	0	0			0	0		
10-15 liters	0	0			7	12.5		
>15 liters	7	12.5			10	17.86		

Table 3: Incidence rate of subclinical mastitis with associated factors at cows and quarters level.

Cow level					Quarter level			
Animal risk factors	**No of animal affected**	**Incidence rate per 100 cows per week**	**(χ^2)**	**P-value**	**No of quarters positive**	**Incidence rate/100quarters per week**	**(χ^2)**	**P-value**
Breed								
Holstein cross	22	1.73	1.06	0.304	88	1.38	4.219	0.04
Jersey cross	6	6.12			24	3.12		
Parity								
1 Calve	3	1.26	1.28	0.733	12	1.14	5.139	0.162
2 Calve	14	1.89			56	1.39		
3 Calve	8	2.19			32	1.69		
4 Calve	3	10.71			12	6.35		
>5 Calve								
Lactations age								
Early	11	1.06	5.18	0.075	44	0.93	20.72	0.001
Mid	11	5.24			44	2.84		
Late	6	2.18			24	2.76		
Age								
<5years	3	0.86	1.01	0.605	12	0.8	4.022	0.134
5 and 6 years	22	2.21			88	1.61		
>6 years	3	10.71			12	6.35		
Milkyield/Litter								
<5	2	0.55	12.46	0.006	8	0.53	49.82	0.001
45056	15	2.16			60	1.55		
45214	9	3.29			36	2.38		
>15	2	4.76			8	3.57		

Table 4: Proportions of identified bacteria from subclinical mastitis at quarter level.

Isolates	No.of isolates	Percentage (%)
Staphylococcus aureus	36	32.14
CNS	18	16.07
Escherichia coli	22	19.64
Micrococcus species	9	8.04
Streptococcus agalactiae	8	7.14
Streptococcus uberis	3	2.68
Streptococcus faecalis	3	2.68
Pseudomonas species	13	11.61
Total	112	100

Discussion

In the present study, from 56 total lactating dairy cattle examined at the beginning of the study, 24(42.9%) dairy cows became positive for both clinical and subclinical mastitis. From 24 positive cows 7(12.5%) cows were affected by clinical mastitis which was similar with the result of [31] who reported 12.5% in Selected Districts of Eastern Hararghe Zone. This result is slightly higher than 4.8%, 3.2%, 5.4% and 5.1% the findings of [2,6,16,30], respectively in different parts of Ethiopia. The difference may be due to greater experience in drying off, the potential effect of level of milking hygiene, herd size, breed difference and the application of sanitary measures in the studied farms [31]. In this study, the prevalence of subclinical mastitis was 30.4%, which is comparable to the result of [6,16,26], who reported 30.1%, 28.34% and 27.3% respectively. It is higher than the finding of [2] which was 23.8% in Shade district, Arsi zone Oromia. This might be due to poor management such as use of common towel cloth, use of common milking machine without proper sanitation of in-between and at the end of milking as which is commonly observable in study area.

The result of the present study revealed that, except breed, all risk factors have a significant association with prevalence of clinical mastitis. Lactation stage and milk yield were found to be having a significant effect on the prevalence of subclinical mastitis. The highest prevalence was recorded in cows with late lactation stage in both cases. Similar report was found that as lactation increases the risk of mastitis increases [27]. The highest prevalence was recorded in cows >6 years aged (8.93%) and in cows having >5 calve (5.36%). The risk of mastitis increases with age and parity number and the higher prevalence in older cows might be due to the fact that older cows have larger teats and more relaxed sphincter muscles that render ease of accessibility of infectious agents in the cows' udder [7]. The highest prevalence was recorded in cows giving milk yield to >15liters (12.5%) when compared to the others for both mastitis. Higher-yielding cows have been found more susceptible to mastitis owing to the position of the teat, and udder susceptible genes making them prone to mastitis and due to less efficacy of phagocytic cells in higher-yielding cows associated with dilution [12].

In the present study (87.5%) cows had subclinical mastitis in four months period of follow up which is comparable to the result of [18] who reported 93.3% and 90.25% for cow-wise and quarter-wise respectively. It is higher than the result of [5]

which was (26.9%), [21] which were 23% and [9] which was 21.9%. The cow level incidence rate of subclinical mastitis in the four months period of follow-up is 2.04; this result was higher than the report of [14], which was 0.49. The highest result of this study may be due to absence of screening and less attention to early management of subclinical mastitis.

Only milk yield was found to have a significant effect on the incidence of subclinical mastitis (p<0.05) at cow level. Higher incidence rate (4.76) was recorded in cows giving milk yield of >15liters as compared to other groups. This confirms the scientific truth reported by [18] who concludes in his findings as mastitis is one of the most prevailing diseases of high-yielding dairy animals. In quarter level breed, Lactation stage and milk yield were found to have a significant effect on the incidence of subclinical mastitis (P<0.05) as indicated in (Table 3).

Cows at mid and late lactation stages had higher incidence rate of mastitis than early stage in this report. This finding agrees with the result of [5] who report higher number of mastitis cases, in mid and late stages of lactation. This might be due to the fact that milk production was highest in second and third lactation [25] and because of inefficient immune system response of the cow due to gradual change of feed formulation to dry cow diet, and stress triggered by advancing gestation [1].

From a total of 128 quarters examined and became positive the relative bacterial isolation from 128 affected quarters were 54(48.21%), 22(19.64%), 14(12.50%), 13(11.61%), and 9(8.04%) for *Staphylococcus* species, *Escherichiacoli*, *Streptococcus* species, *Pseudomonas* species, and *Micrococcus* species respectively. The finding of higher proportion of *Staphylococcus* species from total isolates is in agreement with the report of [7,10,14] who reported the higher result of 52.9%, 30.7%, and 59.26% respectively. The relatively high prevalence of *Staphylococcus species* in this study could be associated with the absence of improper post milking teat dipping, poor udder, and teat washing before milking. This was confirmed by similar report by [10] from selected areas of Bench Maji Zone, Southwest Ethiopia.

In this study *Escherichiacoli* is the second dominant isolate accounted for about 19.64%, of the total isolates and this finding is comparable with the finding of [15] who reported it as the second most isolate. The finding of *E.coli* in this study may be associated with poor hygienic condition in the farm, dirty bedding and contaminated stall where cows are kept due to old dairy housing that is not comfortable for cleaning.

In this study, *Streptococcus* species accounted for 12.5% of the total isolates. This finding was lower than that of [14], which was (23.5%). The lower isolation rate of *Streptococcus* species might be due to widespread use of Penstrip in the study farm for the treatment of mastitis. Eliminating existing infections reduces the exposure of susceptible quarters and may be obtained by treatment during lactation or at dry off, or by culling of the infected animals and again separation of the infected animals from the susceptible group may also be an effective method to limit the exposure of susceptible animals and reduce the risk of new infections.

Conclusion

The result of present study showed that the high incidence rate of subclinical mastitis at cow and quarter levels, which can interfere with the efficiency of milk production and has high economic importance. Milk yield and lactation stages were important risk factors precipitating the occurrence of mastitis. The most important pathogens causing mastitis in the study area were *Staphylococcus* species and *E.coli*, which was isolated from the milk sample in higher proportion. This study was done for four-month which is too short for incidence study in different seasons. Therefore, regular screening of subclinical mastitis for early detection and management, standard milking practices for individual cow, good housing and proper sanitary conditions of cow's environments will improve the current conditions in the farm. Furthermore, antibiotic resistant test and seasonal incidence rate determinations were forwarded as research gap.

Author Statements

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Statement of Animal Rights

Animal handling and sample collections were conducted according to standard guidelines for Animal research ethics, Haramaya University, College of Veterinary Medicine, 2021

Conflict of Interest

The Authors declared that they have no conflict of interest.

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