

A Study on the Prevalence of Subclinical Mastitis in Lactating Cows and Associated Risk Factors in Wolmara District, Oromia Regional State, Ethiopia

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ABSTRACT

A cross-sectional study was conducted to estimate the prevalence and associated risk factors of mastitis in dairy cows. The present study was carried out to investigate prevalence of subclinical mastitis (SCM) in milking cows and risk factors associated with sub-clinical mastitis. A total of 384 lactating cows were randomly selected to identify the SCM from 12 PAS of Wolmera Woreda and cow information was collected from the farmers' thought interview. Milk samples were collected aseptically from California mastitis test (CMT) positive cows. Result showed that 172 lactating cows (44.8%) and 358(93.2%) quarters were positive to CMT. The prevalence of SCM was ($p < 0.05$) significantly high in European crossbreds (56.5%) compared to local breed (36.3%). The highest prevalence found in the age group (4-7) years and the highest prevalence in parity more than 4 year was 37.2%. The prevalence was significantly ($p < 0.05$) higher in cows with earlier lactation (41.3%). This study indicated a higher prevalence of mastitis linked with several risk factors. Thus, early diagnosis and regular screening of cows for subclinical mastitis together with proper therapeutic management of clinical cases are of paramount importance. Moreover, control and prevention strategies should be designed and implemented with great emphasis given to risk factors to reduce bovine mastitis and its impact on milk production and food security.

Introduction

In Ethiopia, livestock represents a major national resource and form an integral part of the agricultural production system. The country has the largest livestock population of any African country with estimated 43.1 million heads of cattle and cows representing the largest proportion of indigenous cattle of the country [1]. Milk produced from these animals provides an important dietary source for the majority of rural as well as a considerable number of the urban and peri-urban population. However, milk production often does not satisfy the country's requirements due to a multitude of factors, out of which disease of the mammary glands known as mastitis is among the various factors contributing to reduced milk production [2]. Mastitis is defined as an inflammation of the parenchyma of mammary gland, which can reduce milk yield and alter milk composition [3]. There are two main classes of mastitis.

The first is clinical mastitis, which manifests signs observed from the animal or the milk. The other is subclinical mastitis, which produces no visible signs from the udder except when using diagnostic tools. Since the quality and quantity of the milk is influenced by mastitis, it is considered to be one of the most important causes of economic losses in the dairy industry worldwide. Mammary gland infections cost the US dairy industry approximately 2 billion dollars annually and have a similar impact in Europe [4].

Somatic cells are part of the natural defence mechanism and include lymphocytes, macrophages, polymorphonuclear cells and some epithelial cells [5]. Somatic cell count (SCC) can be measured quantitatively by California mastitis test (CMT). It is a simple, easy and low-cost screening test for subclinical mastitis at dairy farms. Validity of CMT in diagnosis of infected quarters was established in

various milking stages [6,7]. Mastitis is the most frequent diseases of dairy cow and reduces quality of milk. About 140 to 200 USD / Cow/Year ease next to reproductive disorders and one of the major causes for economy failure in Ethiopia [2]. In Ethiopia mastitis prevalence rate was 85.6% and 81.2% using CMT and somatic cell count (SCC), respectively [8]. An over all of 30.2 and 5.5% for subclinical and clinical mastitis respectively [9]. Most of the previous studies in Ethiopia were concentrated on the investigation of the prevalence and few risk factors for mastitis at cow level and no or little effort has been made to assess the prevalence, management and hygienic practices at herd/farm-level. A focused study on contagious mastitis with emphasis on subclinical type is lacking. Besides, teat morphology, which is inherited anatomical feature of the cow that may affect mastitis occurrence and could serve as a marker trait for selection to reduce mastitis in dairy cattle, is less investigated. It is therefore important to assess the effect of the variable on clinical and sub-clinical mastitis under Ethiopian condition and recommend preventive measures to reduce loss attributed to the disease. Moreover, given the huge economic relevance due to lack of clinical visibility and subsequent effects, investigation of sub-clinical mastitis at herd-level is of paramount importance for designing feasible prevention and control strategy [10,11].

Despite high cattle population and milk production potential of the study area, sub clinical mastitis is insufficiently investigated and information relating to its magnitude, distribution and risk factors is scant. Thus, the objectives of the study will be:

- a. To evaluate the prevalence of subclinical mastitis in apparently healthy dairy cows in Wolmera district.
- b. To evaluate associated risk factors affecting on subclinical mastitis

Materials and Methods

Study Area

The study was conducted in and around Holeta town located 45km away from Addis Ababa in the south west direction, 9° 3' N and 38° 30' E, at an altitude of 2,400m above sea level in central highlands. The area is characterized by mild subtropical weather with minimum and maximum temperature ranging from 2 to 9 °C and 20 to 27 °C, respectively. The area receives annual rainfall of 1060mm [9].

Study Population

A total of 384 dairy cows were examined in different kebeles of Wolmera district such as, (G/kuyu, G/qerans, B/harbu, W/harbu, T/harbu, Shola, Sadamo, Damotu, W/choke, Walmara, Q/odo, B/qorodo, B/2ffaa, Bu/harbu, B/1ffaa). The dairy cows were distributed according to breed (local cows, Holstein Friesian breed, Jersey and Holstein crossed with Borena), age (cows aged less than 6 years young and cow aged greater than or equal to 6 years

old). all dairy cows with no clinical symptoms were examined. All animals were subjected to clinical and physical examinations, with special interest towards the udder and teats. At the time of each examination, the breed of the cow, age of the cow, health status of the mammary glands and the respective kebele were recorded.

Study Design

A cross sectional study was conducted from July 2017 to December 2017. Dairy animals were selected from different kebeles of the district and examined by California mastitis test for the presence or absence of sub clinical mastitis at Wolmera district veterinary clinic.

Sample Size and Sampling Method

Simple random sampling technique was followed to select the study animal, and the desired sample size had been calculated according to the formula given in [12]. Since there is no previous report on this issue 50% expected prevalence is used.

$$n = 1.962P \exp(1 - P \exp) / d^2$$

Accordingly, a total of 384 dairy cows were examined in different kebeles of Wolmera district.

Sample Collection and CMT Test

The udders and especially teats were cleaned and dried before sample collection. Each teat end had been scrubbed with cotton moistened with 70% ethyl alcohol. A squirt 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 the CMT reagent (4% NaOH in distilled water and 1% bromothymol blue) was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane for 15 seconds. Based on the thickness of the gel formed by CMT reagent-milk mixture, test results were scored as 0 (negative/trace), +1 (weak positive), +2 (distinct positive), and +3 (strong positive). Positive CMT-cows were defined as having at least one CMT-positive quarter. The total number of blind teats as well as those with clinical infection was subtracted from the total number of teats and the difference was used to calculate the prevalence of subclinical mastitis.

Physical Examination of Mastitis

Udder attachment, parity number, any physical abnormalities such as swelling of the udder, presence of lesions, anatomical malformations and tick infestation were recorded. The milk was examined for its colour, odour, consistency and other abnormalities.

Data Analysis

The data was compiled and analysed with Statistical Package for Social Sciences (SPSS statistical package version 17). Prevalence estimation of positive cows was determined using standard formulae (that is, the number of positive animals/samples divided by the total number of animals/samples examined). Descriptive

statistics such as percentages and frequency distributions were used to describe/present the nature and the characteristics of the data

Results

Out of 384 examined 172 cow have subclinical mastitis with prevalence of 44.8.

Severity of Subclinical Mastitis on CMT Test

Based on the CMT result 86(50%) cows had weak infection, 76 (44.2%) had distinct positive and 10 (5.8%) cows had strong positive (Tables 1&2).

Table 1: Degree of severity of subclinical mastitis.

CMT Result	Number Positive	Percentage
Weak positive (1)	86	50.0
Distinct positive (2)	76	44.2
Strong positive (3)	10	5.8

Table 2: Prevalence of sub clinical mastitis at quarter levels (N=358).

Quarter Infected	Number Positive	Percent
RF	88	51.1
LF	86	50.0
RH	158	91.9
LH	26	15.1

Prevalence According to Teat Quarter

Out of the examined quarters no teat was found to be blind. The quarter level prevalence of sub clinical mastitis from the highest to lowest rate of infection were right front teats (RF) 88 (51.1%), left hind teat (LH) 26 (15.1%), left front (LF) 86 (50%) and hind right teats (HR) 158 (91.9%) respectively (Table 3).

Table 3: Prevalence in different kebeles (n=384).

Peasant Association (PA)	Number Examined	Number Positive	Percent (%)
Bu/Harbu	68	34	50
Ba/1ffaa	13	6	46
B/Qorodo	10	5	50
Damotu	11	7	63.6
G/kuyu	47	23	48.9
G/Qerans	26	13	50
Q/Odo	11	6	54.5
Sadamo	42	16	38
Shola	24	6	25
T/Harbu	78	38	48.7
W/Choke	14	10	71.4
Walmara	40	14	35

Prevalence in different kebeles

From the twelve kebeles W/choke (71.4%) had the highest prevalent sub clinical mastitis case with number of animal positive

of 10 from 14. And Sholahave lower sub clinical mastitis case with number of animal positive of 6(25%) of animals (Table 3).

Different Risk Factors

According to different risk factors categories the highest prevalence was recorded in cross breed, young age group, fourth parity, and first lactation stage (Table 4). There was a statistically significant association (P<0.05) between the infection of sub clinical mastitis and breed, age group, parity, lactation stage, teat and barn hygiene, mammary gland cleaning and teat deep (Table 5).

Table 4: Prevalence of subclinical mastitis according risk factors.

Breed	Number Examined	Number Positive	Percent
Local	223	81	47.7
Cross	161	91	52.9
Age			
Young (4-7)	321	124	72.1
Old (>8)	63	48	27.9
Parity			
First	6	12	7
Second	125	34	19.8
Third	74	40	23.3
Fourth	95	64	37.2
Fifth	25	17	9.9
Sixth	6	5	2.7
Lactation Stage			
First	178	7	41.3
Second	93	38	22.2
Third	113	63	36.7
Teat Hygiene			
Good	82	20	11.6
Mild	220	93	54.1
Poor	82	59	34.3
Barn Hygiene			
Good	80	17	9.9
Mild	245	110	64
Poor	59	45	26.2
Teat Deep			
After	4	2	1.2
Before	222	79	45.9
Non	158	91	52.9
Mammary Gland Cleaning			
Before	311	112	70.9
After	1	0	0
Non	72	50	29.1
Hand Wash During Milking			
Washed	345	140	81.4
Non	39	32	18.6

Table 5: Prevalence of subclinical mastitis against the associated variable.

Breed	No. Examined	No. Positive	Percent	p-value	χ^2 value
Local	223	81	36.3	15.426	0.000
Cross	161	91	56.5		
Age					
Young (4-7)	321	124	40	74.349	0.000
Old (>8)	63	48	60		
Parity					
First	6	12	20	62.819	0.000
Second	125	34	27.2		
Third	74	40	54.1		
Fourth	95	64	64.7		
Fifth	25	17	70.8		
Sixth	6	5	83.3		
Lactation Stage					
First	178	7	40.1	9.758	0.045.
Second	93	38	40.9		
Third	113	63	58.9		
Teat Hygiene					
Good	82	20	24.4	41.024	0.000
Mild	220	93	42.9		
Poor	82	59	72.0		
Barn Hygiene					
Good	80	17	21.2	41.574	0.000
Mild	245	110	44.9		
Poor	59	45	76.3		
Teat Deep					
After	4	2	50	19.683	0.000
Before	222	79	36.1		
Non	158	91	57.6		
Mammary Gland Cleaning					
Before311	311	112	39.2	22.399	0.000
After1	1	1	0		
Non72	72	50	69.4		
Hand Wash During Milking					
Washed	345	140	40.6	25.370	0.000
Non	39	32	82.1		

Discussion

In Ethiopia, subclinical mastitis is considered to be an important challenge for the dairy development. This study also indicates subclinical mastitis to be the major problem in the study area. In the present study, the overall prevalence of sub clinical mastitis is 44.8% at cow level. This finding is almost similar to finding of [13] who reported from 262 cows examined, 105 (40.1%) and from 1048 quarters examined, 170 (16.1%) were positive for sub-clinical mastitis using CMT IN in Bishoftu Town and lower in comparison with finding of [14], who reported that the overall mastitis prevalence in the farm was 66.6% in Assella Dairy Farm in

Oromia Region, Ethiopia which could be due to lack of awareness, housing and milking practices they are accustomed in the area. Subclinical mastitis is a complex disease and the prevalence could be affected due to variation in management system, age, milk yield, body condition, parity, environment and other conditions. Thus, these factors might have contributed to the observed differences in prevalence of subclinical mastitis.

The risk factor considered for this study was breed, age group, parity and lactation stage. In the present study from selected potential risk factors breed, age, stage of lactation, and parity had statistically significant effect ($P < 0.05$) on the occurrence of mastitis

which agrees with the report by [15]. Similarly, the environment risk factors considered for this study were teat and barn hygiene, mammary gland cleaning and teat deep. The occurrence of mastitis based on these risk factors showed statistically significant effect with the occurrence of sub clinical mastitis. This was also reported by several investigators to have association with the occurrence of sub clinical mastitis [16-19]. The study showed that there were significant statistical associations ($p < 0.05$) with the prevalence of mastitis and the parity number of animals; cows with many numbers of calves were with higher prevalence of mastitis and the risk of subclinical mastitis increases with increasing parity number which agrees with the finding of [19-22] who found that the risk of clinical and subclinical mastitis increase significantly with increasing parity number of the cow.

The higher prevalence in cows at three and above calved could be due to increase ease of penetration of the teat duct by pathogens and accumulated previous infection [23]. It is postulated that younger animal is less susceptible; through a more effective host defence mechanism. Older cows, especially after four calving are more prone to mastitis [24]. The present study indicated sub clinical mastitis is a prevalent disease in lactating cows in Wolmera district. Breed, age, parity, and management system are the main factors that were associated with mastitis. The subclinical cases are by far more frequent than the clinical ones. Unequivocally, silent loss of milk production due to subclinical mastitis along with blind teats could cause adverse effect on the dairy activity of the study area that guarantees serious attention in creating awareness and prevention strategy. Ethiopian dairy farmers are not well informed about the invisible loss from subclinical mastitis and thereby there is no practice of subclinical mastitis screening tests. In conclusion, in Ethiopia, the subclinical form of mastitis received little attention and efforts have been concentrated on the treatment of clinical mastitis, however high economic losses often come from subclinical mastitis [24,25].

Authors Contributions

BG: participated in the design of the study, conducted laboratory work, involved in data analysis and manuscript preparation. DA: participated in the design of the study, involved in data analysis and manuscript preparation. Both authors read and approved the final manuscript.

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Availability of Data and Materials

All relevant data supporting the conclusions of this article are contained within the article.

Ethics Approval and Consent to Participate

The protocol was approved by the ethical review board of the School of veterinary medicine, Wollega University. Informed written consent was taken from all participants prior to participation in this study.

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