Clinical and Subclinical Bovine Mastitis and Associated Risk factors in Small-scale Dairy Farms in Bahir Dar and its envirion, Amhara region

Mussie Hailemelekot (musiehailu@yahoo.com)
Bahir Dar University College of Agriculture and Environmental Sciences
Melaku Chalachew
Amhara Region Livestock Development and Promotion Agency
Sam Laiju
Bahir Dar University College of Agriculture and Environmental Sciences

Research article

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Abstract

Background

Cross-sectional study was conducted from June 2017 to January 2018 with the objective of determining the prevalence of mastitis and identify the associated risk factors in and around Bahir dar dairy farms. Totally 302 lactating cows were selected using simple random sampling method. Initially, mastitis cases were diagnosed based on California Mastitis Test (MCT) results and the nature of gel formation. Then positive samples were subjected to bacterial culture and isolation using blood agar, MacConkey agar, colony morphology, Gram stain, and biochemical tests. Statistical analysis was carried out using STATA software and association of mastitis with risk factors was analyzed using chi square test. The association was considered as significant when p-value was less than 0.05.

Result

The overall cow level prevalence of mastitis using CMT was 25.16% (76/302) with clinical and sub clinical mastitis of 3.64% and 21.52%, respectively. Similarly, the quarter level mastitis was 10.43% (126/1208) with clinical and sub clinical cases of 1.49% and 8.94%, respectively. Bacteria identified were *Staphylococci Spp, Escherichia coli, Bacillus Spp*, and *Streptococcus Spp*. with a proportion of 66.07% .94%, 8.0% and 7.14%, respectively. Body condition score, cow Hygiene, Parity, history of mastitis, stage of lactation, teat lesion, use of same clothing, lactating mastitis positive animal last were found to be significant risk factors.

Conclusion

The current study revealed moderate prevalence of mastitis warranting application of appropriate hygienic practices during milking and awareness creation to the dairy farmers.

Background

Ethiopia enjoys diverse topographic and climatic conditions and milk production takes place across all agro-ecological zones(1) In the highlands, milk is mainly produced by small-scale mixed farming, while in the lowlands, pastoralist production systems are predominant There are also intensive and commercial dairy farms concentrated in and around major cities and towns of the country(1) About 98.20 percent of the total cattle in the country are local breeds, remaining are cross and exotic breeds that accounted for about 1.62 percent and 0.18 percent, respectively (1). Market oriented urban and peri-urban dairy production systems, based on up-graded dairy stock and purchased conserved feeds are emerging and dominating in most urban centers(2) The systems involve the production, processing and marketing of milk and milk products that are channeled to consumers in urban centers with a number of beneficiaries along the value chain (2).

Market-oriented urban and peri-urban dairy production systems have tremendous potential for development and play a significant role in minimizing the acute shortage of milk and dairy products in urban centers(3) There is also a strong rural-urban linkage in these systems in terms of supply of labor, feeds, water and manure (3).

Mastitis is a complex and costly disease of dairy cows, that results from the interaction of the cow, environment including milking machine and microorganism (4). Risk factors associated to clinical mastitis are milking routine, type of housing, feeding, and season, as environmental effects. In addition, older cows, later first calving, first stages of lactation and cows with deep udders, week attachments, and high production are more liable to mastitis.

Many infectious agents have been implicated as cause of mastitis in cattle. The most common organisms being *Streptococcus agalactiae* and *S. aureus* whereas, environmental mastitis is associated with Coliforms and environmental Streptococci are also frequently found in the cow's environment (5). Mastitis causes a reduced milk production, not only at the occurrence of the mastitis but throughout the rest of the lactation, increases the risk of new cases of mastitis and increases the risk of culling (6).

Clinical and subclinical mastitis both severely affect milk yield and milk quality. Milk production of cows bearing mastitis is significantly lower than that of healthy cows. Furthermore, the nutritional quality is lower and the somatic cells count (SCC) is substantially higher (7). The SCC of milk is regarded as the industry's standard indicator for the general quality of produced milk. It is determined as the total count of white blood cells per milliliter of milk. Normal milk is believed to have SCC of approximately 200,000 cells/ml or less. An infection in the mammary gland of the udder causes a large influx of somatic cells, predominantly polymorphonuclear neutrophils, which can increase the SCC of milk up to 1 million cells/ml(8). Subclinical mastitis commonly contributes a more substantial part to high SCC's within a herd and is usually a reliable indication of the development of clinical mastitis (9).

According to (10*)* mastitis is one of the most significant health problems of dairy herds as it causes physical, chemical and bacteriological changes in the milk of dairy animals resulting in inferior quality and quantity of produced milk with possible public health importance. Therefore, conducting research on its prevalence and associated risk factors will contribute a lot to design appropriate preventive measures in dairy farm. Moreover, conducting researches on this regard has a paramount importance since the problem is a bottle neck to the production performance. Therefore, the aim of this study was determining the prevalence of mastitis and associated potential risk factors in and around Bahir dar dairy farms.

Methods

Description of Study Area

The study was conducted in and around Bahir-dar from June 2017 to January 2018. Bahir Dar is the capital of the Amhara National Regional State (ANRS) of Ethiopia. It is located at $11^0$38'N, $37^0$10'E on the

southern side of Lake Tana where Blue Nile river starts. As of the relative location, it has a road distance of 565 km from Addis Ababa capital of Ethiopia. It is located at an altitude of 1800 m a s l with average annual temperature and rain fall of 19.6^{o}c and 1419 mm, respectively. The city is situated at the southern shore of Lake Tana: a freshwater lake with weak seasonal fluctuation having 3156 km2 area which is the largest lake in Ethiopia and the third among those in the Nile Basin (11).

Study Population

The study populations were all lactating Holstein-zebu crossbred cows in and around Bahir dar. According to Bahir dar City Administration Office of Agriculture, there are 84 small holder dairy farms containing 977 dairy cows kept in exclusive stalls and provided with supplementary diets in addition to hay and agricultural by-products. Out of which 632 were lactating cow in the studied farms. The study animals were selected from small holder dairy farms using lottery method in the above indicated population.

Study design

A cross-sectional study type was carried out to investigate the prevalence of clinical and sub-clinical mastitis at cow and quarter levels and to detect possible association between various risk factors and the occurrence of mastitis.

Sample Size Determination and Sampling Techniques

The sample size was determined at 95% confidence interval, 5% absolute precision and from previous studies in the study area (12), with an expected prevalence of 26.9%.Thus, the sample size calculated by using (13) formula to be 302 animals.

$$n = \frac{1.96^2 * P\,exp\,(1-Pexp)}{d^2}$$

Where n = the total sample size

P exp = expected prevalence

d = absolute precision.

The household or owners list was recorded and taken as a sampling frame simple random method was used to select the dairy farm and study animals.

Study Methodology

The study methodology involves reviewing farm documents, farm inspection, animal examination and laboratory investigation. Farm records with respect to animals' parity, stage of lactation, past disease history, production performance were reviewed.

Farm inspection

Farm inspection was carried out to assess the housing conditions, feeding practices and milking practices. The housing conditions was graded as poor when there is bad smell, feed trough and gutter (waste drainage) were dirty, animals flank, udder and belly were soiled. The housing conditions were graded as good when none of the above indicated defects were observed (14).

Animal Examination

Lactating study cows were examined to determine their body condition, presence or absence of tick and teat lesion. Body condition scoring was assessed using 1–5 point scale based on palpation of back bone and lumbar process and evaluation of coverage of fat and muscle (15). Hygiene scoring of cows was determined based on a scale of 1–4 for three zones of the body; udder, lower leg and upper leg and flank (16). Morphology and udder attachment were first examined visually and then through palpation for possible fibrosis, cardinal signs of inflammation, visible injury, and atrophy of the tissue and swelling of the supra-mammary lymph nodes. The size and consistency of mammary gland were inspected for any abnormalities, such as asymmetry, swelling, firmness, and blindness. Physical appearance of milk secretion from each mammary quarter was also examined for the presence of clots, flakes, blood and watery secretions. Presence of hotness, redness and painful sensation also detected by inspection and palpation.

Preparation of udder and teats for milk sample collection

The udder, especially the teats were cleaned and dried before milk sample collection. Dust, particles or other filth were removed by brushing the surface of the teats and udder with a dry towel. Then washed with tap water and disinfected with cotton soaked in 70% ethyl alcohol.

Sampling method and Sample handling

Milk sample was collected by standard milk sampling techniques from all lactating cows. To reduce contamination of the teat ends during sample collection, the near teats were sampled first followed by the far once. Approximately 20 ml of milk was collected from each quarter for CMT test into labeled sterile screwed cap universal bottle after discarding the first three milking streams. Samples were placed in ice box and transported to Bahir dar Animal Disease Surveillance, Investigation and Diagnostic Laboratory department of microbiology and serology and processed as soon as possible without any delay.

California Mastitis Test (CMT)

Mastitis cases was diagnosed based on CMT results and the nature of gel formation (milk and CMT reagent), which shows the presence and severity of the infection. Before sample collection for bacteriological examination, milk samples were examined for visible abnormalities and screened by the CMT. According to (5) from each quarter of the udder, a squirt of milk sample was dropped in each of the strip cups on the CMT paddle and an equal amount of 3% CMT reagent was added to each cup and mixed gently. The test result was interpreted based on the thickness of gel formed by CMT reagent and

milk mixture and score as 0(negative), T (trace), 1(weak positive), 2(distinct positive) and 3(strong positive).Finally quarters with CMT score of 1 or above was judged as positive for mastitis; otherwise negative (5).

Table 1
Interpretation CMT findings

Score	Interpretation	Visible reaction
0	Negative	Milk fluid and normal
T(Trace)	Trace	Slight precipitation
1	Weak positive	Distinct precipitation but no gel formation
2	Distinct positive	Mixture thickens with gel formation
3	Strong positive	Viscosity greatly increased strong gel i.e. cohesive with a convex surface
Source: (5).		

Culture and Isolation of bacteria

In the laboratory a loop full of the milk sample was streaked on blood agar base enriched with 7% sterile sheep blood and MacConkey agar and incubation was made at 24 to 48 hours. Then plates were examined for any growth, colony morphology and hemolytic characteristics on blood agar. Subculture was also done to obtain pure isolates for further identification. Culture positive plates were identified according to gram stain reaction and colony morphology, biochemical tests. Identification of bacterial species for Staphylococci, Streptococcus species, Bacillus species and *Escherichia coli* were done according to (5) using standard methods.

Data Entry and Analysis

Data was coded, cleaned and entered into Microsoft Excel. Statistical analysis was carried out using STATA. Data was analyzed descriptively using descriptive statistics in the first step; thereafter association of the different variables with interest of outcome was analyzed using chi square. The association was considered as significant when p-value was less than 0.05.

Results

Prevalence of Mastitis

A total of 302 dairy lactating cows were examined for the presence of mastitis from June 2017 to January 2018 in and around Bahir dar. The overall cow level prevalence of mastitis using CMT was 25.16% (76/302) with clinical and sub clinical prevalence of 3.64% and 21.52%, respectively. Similarly, the quarter level prevalence was 10.43% with clinical and sub clinical mastitis of 1.49% and 8.94%, respectively (Table 2).

Table 2
Clinical & subclinical mastitis in lactating dairy cows in and around Bahir dar, 2017/18.

Category	Total no of examine	Total no of positive	Prevalence
At cow level			
Clinical	302	11	3.64%
Sub Clinical	302	65	21.52%
Overall	302	76	25.16%
At quarter level			
Clinical	1208	18	1.49%
Sub Clinical	1208	108	8.94%
Overall	1208	126	10.43%

Bacteriological Examination

From a total of 126 milk sample taken from 76 affected lactating cows, 112 were culture positive, 12 were rejected due to contamination and 2 were without any bacterial growth. Different pathogens were identified as a cause of mastitis including *Staphylococci Spp, Escherichia coli, Bacillus Spp*, and *Streptococcus Spp*. *Staphylococci species* 66.07% (74/112) were the dominant pathogen took the lion share followed by *Escherichia coli* 16.94% (21//112) (Table 3)

Table 3
Bacterial isolates from mastitis positive cows in and around Bahir dar, 2017/18

Types of bacteria isolated	Frequency	Percentage (%)
Staphylococci species	74	66.07%
Escherichia coli	21	16.94%
Bacillus species	9	8.03%
Streptococcus species	8	7.14%
Total	112	100%

Potential Risk Factors for occurrence of Mastitis

The result of statistical analysis indicated that body condition, cow's hygiene, parity, stage of lactation, history of mastitis, the presence of teat lesion ,using same clothing to clean udder and milking mastitis cow last were the factors the yielded significant association to the development of mastitis.

The current study indicated that the prevalence of mastitis in relation to body condition was higher in poor body condition compared to those cows with good body condition. There was statistically significant difference among body condition (x^2 = 5.25; P = 0.022).The prevalence of mastitis in cows

having poor hygienic condition was significantly high (57.8%) than clean condition (8.5%). The likelihood of mastitis was significantly higher in poor hygienic condition ($x^2 = 87.33$; $P < 0.001$) (Table 4).

Regarding to parity, significant higher prevalence of mastitis was observed in multiparous parity (57.14%) than primiparous lactating cows (6.32%) ($x^2 = 96.66$; $P < 0.001$). The previous exposure to mastitis was another variable that showed association to mastitis. The prevalence of mastitis was significantly higher ($x^2 = 217.71$; $P < 0.001$) in cows with the history of mastitis than cows that had not. Similarly, cows with early lactation stage showed highest mastitis prevalence (36.45%) than mid and late stage of lactation with the prevalence of 26.25% and 13.91%, respectively. The analysis indicated that there was highly significant difference among different stages of lactation ($x^2 = 15.01$; $P = 0.001$). Although slightly high number of mastitis cows were found to have pendulous teats than small teats there was no significant difference between them (Table 4).

Table 4. Host related risk factors and occurrence of mastitis in lactating cows in and around

Bahir dar, 2017/18.

Variables	No of cows examined	No of positive cows	Prevalence (%)	Chi square X^2	P-Value
Body condition score				5.26	0.022*
Poor	243	68	27.98%		
Good	59	8	13.56%		
Cow Hygiene				87.33	0.000*
Clean	200	17	8.5%		
Dirty	102	59	57.84%		
Parity				96.66	0.000*
Primiparous	112	64	57.14%		
Multiparous	190	12	6.32%		
Mastitis history				217.71	0.000*
No	218	5	2.29%		
Yes	84	71	84.52%		
Stage of lactation				15.02	0.001*
Early	107	39	36.45%		
Mid	80	21	26.25%		
Late	115	16	13.91%		
Teat type				0.014	0.907
Small	97	24	24.74%		
Pendulous	205	52	25.37%		

Lactating cows with teat lesion were highly affected by mastitis than those animals without teat lesion. The difference in presence and absence of teat lesion was significant ($x^2 = 185.27$; $P < 0.001$) to the occurrence of mastitis. But in the current study, the presence of tick did not show any significant association to the prevalence of mastitis (Table 5).

From the current study it was evident that higher number of cows were affected by mastitis in those farms not practicing washing of udder and don't dry the udder before milking than those who are washing and drying the udder before milking. However, there was no significant difference between them (Table 5). The occurrence of mastitis was higher in lactating cows in which the owner's utilized same clothing to dry the udder than those who did not ($x^2 = 5.82$; $P = 0.016$). The prevalence of mastitis was significantly lower in lactating cows from dairy farms practicing milking of affected cow last than those who didn't do this practice ($x^2 = 12.38$; $P = 0.001$) (Table 5).

Table 5. Teat lesion, teat type and teat washing versus occurrence of mastitis in and around

Bahir dar, 2017/18.

Variables	No of cows examined	No of positive cows	Prevalence (%)	Chi square X^2)	P-Value
Teat lesion				185.27	0.000*
No	211	6	2.84%		
Yes	91	70	76.92%		
Presence of tick				0.13	0.909
No	229	58	25.33%		
Yes	73	18	24.66%		
Udder washing befo milking				0.035	0.852
No	34	9	26.47%		
Yes	268	67	25%		
udder Drying after washing				0.711	0.399
No	111	31	27.93%		
Yes	191	45	23.56%		
Use same cloth to all animals					
No	93	15	16.12%	5.8	0.016*
Yes	209	61	29.19%		
Milking mastitic cow last				12.38%	0.000*
No	162	54	33.33%		
Yes	140	22	15.75%		

Discussion

The overall prevalence of mastitis in the current study at cow level was found to be 25.16%. This is comparable with the result of (17), who recorded an overall prevalence of 28.2% at Bahir Dar and its surroundings. But it is a bit lower than the report of (18) 34.9% in the southern Ethiopia. The present study was very low when compared with the work of (19, 20), who reported a prevalence of 64.4% in Asella and 52.78% in and around Sebeta, respectively. The variation could be attributed to management differences, breed considered and agro-climatic condition.

The overall prevalence of mastitis at quarter level was found to be 10.43%. This quarter level mastitis prevalence was in agreement with (21) who reported 10% dairy farms of Holleta town and (22) reported 13.6% in small-scale dairy farms of Adama town. However, it was higher than the result of (23) who reported 2.4% in small-scale dairy farms of Selalle and lower than the report (24) 29.0% in Adama dairy

farms. Differences in husbandry practices like management practices and other interventions between different areas might be the reason to difference in prevalence reported by various researchers.

The prevalence of clinical and sub clinical mastitis recorded at cow level in the present study was 3.64% and 21.52%, respectively. This finding is comparable with the report of (17), who reported clinical prevalence of 3% and subclinical cases of 25.2% at Bahir Dar and its surroundings. However, it is a bit lower than the findings of (25), who recorded 5% clinical and 32. 2% sub clinical cases in the urban and peri-urban dairy farms at Addis Ababa, central Ethiopia. The results of both clinical and subclinical mastitis in this study are much lower than that of previous reports (12, 18, 19, 20, and 26). This could be attributed to better attention and well informed of dairy holders about the effect and consequence of mastitis form time to time.

In the current study, the dominant and most prevalent bacteria identified during bacteriological examination were S*taphylococci* species followed by *Escherichia coli.* This finding is in line with the report of various researchers (12, 20, 21, 22, and 23). The dominance of this pathogen may be ascribed to the lack of proper milking procedure for instance absence of pre and post teat dipping using antiseptics, washing of milker's hands and using teats secretion as a lubricant of teats at the time of milking which is often practiced in the study area might have contributed to the spread of these pathogens from the milker's and infected teats to healthy ones.

In the present study, the environmental bacteria *Escherichia coli* were isolated in high proportion 16.94%.This is in agreement with the reports of (20, 22) who found this isolate in high proportion. The presence of environmental bacteria might be an implication of unhygienic milking practice and contamination of cows' teats and environment with their dung in the study area.

The prevalence of mastitis was higher in poor conditioned cows then good once. This finding is in agreement with reports made by different authors in different parts of the country (22 and 27) who found body condition as one of associated risk factors to mastitis. Animal with poor body condition might experience their immune system not functioning well, thus making them more susceptible to mastitis.

Prevalence of mastitis was significantly associated with milking hygienic practice. Cows

with poor milking hygiene standard are severely affected than those with good milking hygiene condition. This finding is supported by (20, 28, 29) who evidenced the association of hygienic condition to the occurrence of mastitis. This might be due to absence of udder washing, milking of cows with common milkers' and using of common udder cloths, which could be vectors of spread especially for contagious mastitis.

Multiparous cows showed higher prevalence than primiparous cows. This is in agreement with (12, 17, 18, 20, 27, 30) and) who identified parity as risk factor to mastitis in the study conducted at different parts of Ethiopia. As animals produce more and more calves there is risk of contracting bacteria responsible for mastitis and as age goes one the level of immunity reduces

Lactation stage was found to be a risk factor to mastitis and the prevalence was highest in early lactation than mid and late stage of lactation. The result of the current study is in line with (18) who reported mastitis prevalence was higher in early lactation. But it disagrees with the finding of (12, 23) who reported prevalence of mastitis was higher in late stage of lactation. The variation could be attributed to different management practiced in different study areas. The highest prevalence during the early lactation is an indication of infection, probably prior to freshening. It may also be reflection of important changes that occur prior to parturition period in endocrine, nutritional and metabolic status which compromise the immunity of the cow (31).

More cows which had experienced mastitis problem before were found to be more positive to mastitis at current investigation than non-exposed ones. This is comparable with the findings of (18, 22) who indicated cows with previous exposure to udder infection were more likely to be re-infected than those never exposed. This might be attributed to possibility of previously exposed cows remained at carrier state and inefficacy of drugs used for mastitis treatment in the study area.

The presence of teat lesion was found to be one of the risk factors to the development of mastitis. Cows with teat lesion showed significantly higher prevalence of mastitis than those without lesion. This is in agreement with report of (20, 30). This might be attributed to more exposure to infection due to more contact with contaminated environment that make the lactating cows to be prone for mastitis.

Prevalence of mastitis was significantly associated with cleaning practice. The rate of mastitis was higher in lactating cows in which the owner's utilized same clothing to clean all udders than those not doing. This is in line with (32, 33) who reported the same scenario. This might be due to milking of cows with common milkers' and using of common udder cloths, which could favor the proliferation and transmission of mastitis causing organisms.

Milking of infected cow teat last was associated to lower prevalence of mastitis. The infection rate was significantly lower in lactating cows from dairy farms practicing lactation of affected cow last than those not practicing it. The findings of the present study complys with (17) who support the prevention effect of milking affected cows and teat last.

Conclusion And Recommedations

This study was conducted with the aim of determining the overall prevalence of clinical and sub clinical mastitis at cow and quarter level, identification of major pathogens and associated risk factors in and around Bahir dar. The prevalence of clinical and sub clinical mastitis at cow level was found to be 3.64% and 21.52%, respectively with an overall prevalence of 25.16%. Similarly, the overall prevalence of mastitis at quarter level was 10.43. Of which 1.49 was clinical and 8.94 was a sub clinical case. Among the different pathogens involved, *Staphylococcus* species was the dominant pathogen that shared about 66.07% followed by *Escherichia coli* 16.94%. This indicated that contagious and environmental mastitis pathogens were responsible for causing mastitis which suggested improper milking procedures experienced in the farm and contamination of mammary gland with the environment. Among assessed

potential risk factors to the prevalence of mastitis; higher prevalence of mastitis was recorded in poor body condition, bad cow's hygiene, multiple parity, early stage of lactation, previous exposure of mastitis, presence of teat lesion, using same cloth to clean udder and not milking mastitic cow last. This prevalence implied that mastitis is most serious health problem of dairy cows having an adverse effect on productivity of dairy industry of the study area and to the country at large. Based on the above findings the following points are recommended: awareness should be created to the dairy community about mastitis, on hygienic milking methods, housing and environmental sanitation, early screening of cows for subclinical mastitis and culling of old aged and repeatedly infected cows.

Abbreviations

CSA Central Statistical Agency

OR Odd Ratio

m.a.s.l Meter above sea level

Declarations

Ethics approval and consent to participate

Not applicable the experiment was done with the farmer's animal and oral consent was made with the owners

Availability of data and material

Not applicable

Consent of publication

Not applicable

Competing Interests

There is no 'Competing Interests'

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Authors' contribution

Melaku Chalachew = conceived the study, data collection and drafted the manuscript, Mussie

Hailemelekot, Sam Laiju = supervision, analysis and editing of the final manuscript.

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