AgriNat Green Journal of Debre Berhan University, 2025, 1(2), 37-51

Journal homepage: www.journals.dbu.et

Prevalence of Bovine Mastitis and Its Risk Factors in Dairy Farms of Debre Berhan Milkshed, Central Highlands of Ethiopia

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Article information: Received 24 July 2025; Revised on 25 October; Accepted on 25 October 2025

Abstract

The study aimed to evaluate the prevalence of bovine mastitis and its associated risk factors across various production systems in the urban and peri-urban areas of the Debre-berhan Milk shed, situated in the Central Highlands of Ethiopia. A total of 175 households were surveyed via semi-structured questionnaire to identify potential risk factors for mastitis, and clinical examinations and California Mastitis Test (CMT) screenings were performed on 510 lactating cows to determine the prevalence of subclinical mastitis. A cross-sectional study was carried out to assess the prevalence of bovine mastitis and identify its predisposing factors. Mastitis prevalence was assessed in both urban and peri-urban production systems. The findings indicated a significantly higher prevalence in peri-urban systems (62.0%) compared to urban dairy production systems (47.5%). The overall prevalence of mastitis at the cow and quarter level was 72.2% and 55.95%, respectively. Among the mastitis cases, 54.9% were subclinical, while 17.3% were clinical mastitis. Additionally, out of the 2040 quarters examined, 55 (2.65%) were found to be blind. Factors such as age, breed, history of mastitis, milk yield, herd size, and teat lesions showed statistically significant associations with clinical mastitis prevalence (P<0.05). The most commonly isolated pathogen from mastitis-positive cases was Staphylococcus species (57.7%). Overall, the study revealed a high prevalence of mastitis, with age, breed, milk yield, lactation stage, teat lesions, herd size, and barn hygiene being significant contributors. To address this issue, it is crucial to increase awareness among dairy cow owners about maintaining clean udders, improving floor conditions through regular cleaning, and implementing medical interventions to enhance mastitis prevention and control programs in the study areas.

Keywords*: clinical mastitis, risk factors, subclinical mastitis, prevalence*

1. INTRODUCTION

Ethiopia's livestock sector plays a vital role in the country's economy, significantly contributing to its ongoing economic growth. According to the CSA (2021), the cattle population is estimated to be approximately 70 million. The livestock industry is a crucial component of the national economy, accounting for up to 40% of agricultural GDP, over 20% of total GDP, and 20% of the country's foreign exchange earnings (Management Entity, 2021). Within this sector, the dairy industry is particularly important, helping to alleviate poverty, enhance nutritional standards, and improve household incomes. However, various challenges have hindered its full development and promotion, including outdated livestock farming methods, a shortage of professionals in dairy technology and marketing, inadequate distribution networks, and insufficient health programs for dairy cows (CSA, 2007).

Dairy cattle were affected by several diseases such as bovine tuberculosis, brucellosis, and mastitis, which are linked to poor husbandry and management practices. Mastitis, in particular, is a significant concern, as it negatively affects dairy cattle, lowers milk yield and quality, and increases treatment expenses for farmers (Moges *et al*., 2011). This disease is complex, involving a combination of management approaches and infectious agents, with varying causes, intensities, durations, and lasting effects. Economically, mastitis imposes a substantial burden on dairy production and remains a major challenge for the global dairy industry (Lidet *et al*., 2013). Similarly, research by Kebede and Weldemariam (2015) identified mastitis as one of the most prevalent diseases among Ethiopian dairy cows. Studies conducted across different regions of Ethiopia reveal significant variations in mastitis prevalence, influenced by farm type, cattle breed, and management practices. Reported prevalence rates range from 0.93% to 25.1% for clinical mastitis and between 15% and 56% for subclinical mastitis (Belay *et al*., 2022; Deng and Asebe, 2015; Zenebe *et al*., 2014), with the highest prevalence (74.7%) recorded in central Ethiopia, particularly in Addis Ababa (Zeryehun *et al*., 2013).

Despite the widespread and serious impact of bovine mastitis on farmers, research on its distribution and risk factors remains inadequate in certain regions of Ethiopia (Nurye *et al.,* 2021). In order to develop effective strategies for its prevention and control, epidemiological studies examining its prevalence, contributing factors, and associated microorganisms are essential. Although the study area is a major hub for milk production with a substantial population of crossbred dairy cattle, the study area has faced limited research on the prevalence of bovine mastitis.This study, therefore, seeks to investigate the prevalence of mastitis and its risk factors in the Debre Berhan milk shed, located in the North Shewa zone of Amhara Regional State, Ethiopia.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

A cross-sectional study was carried out in the Debre Berhan Regiopolitan City (DRBC) Administration, located in Ethiopia's central highlands (Figure 1). Serving as the administrative and commercial hub of the North Shewa Zone, this city is situated 130 km northeast of Addis Ababa along the paved highway leading to Dessie. The primary agricultural system in the region is crop-livestock farming, with various livestock species such as cattle, sheep, goats, donkeys, horses, mules, and poultry Cattle production, including both indigenous and crossbred animals, is the dominant livestock sector, followed by sheep farming (Hassen *et al.*, 2010).

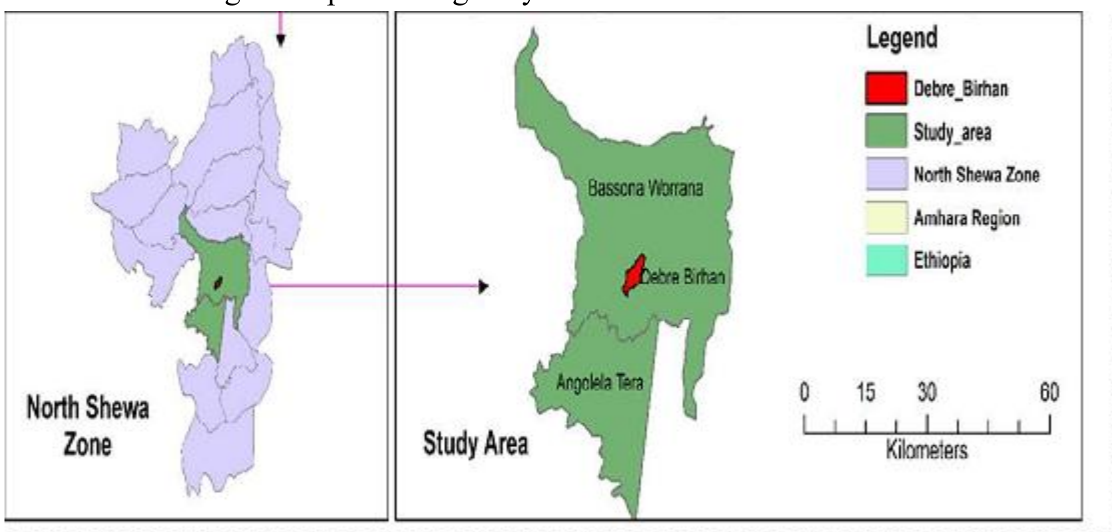

Fig 1. Map of the study areas

2. 2. Study population and Sample size determination

The study included 160 randomly selected dairy farms from the study areas. A total of 510 lactating dairy cows of various breeds and age categories, managed under intensive, semi-intensive, and extensive systems, were examined. The study animals were primarily cross-bred, Jersey, and indigenous local zebu lactating cows, raised in small, medium, and large-scale dairy farms. The sample size was calculated following Thrusfield (2007), applying a 95% confidence interval and a 5% desired precision. Given the absence of prior research in the area, an expected prevalence of 50% was used as the basis for estimation.

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

Where Pexp = expected prevalence, d= absolute precision, n =sample size.

As a result, the calculated sample size was 384 lactating cows. However, the final selection of herds and cows depended on accessibility and the farmers' willingness to participate in the research. To enhance precision, a total of 510 lactating cows were included in the study.

2. 3. Sampling strategy

Eight large dairy farms, 34 medium-sized farms were purposively selected due to the limited number of such farms in the study area, while, 118 smallholder farms were randomly selected for the study. The selection of dairy farms was based on the willingness of the farmers or farm owners and the availability of lactating cows. Lactating cows on each farm were selected purposively. For the purpose of this study, dairy farms were categorized based on the number of cattle the farms have: small-scale farms (1–10 cattle), medium-scale farms (11–20 cattle), and large-scale farms (≥21 cattle). This classification is consistent with previous studies in Ethiopia, such as Megersa *et al*. (2011) and Tschopp *et al.* (2021), which utilized similar herd size categorizations to analyze dairy production systems in different regions of the country.

2. 4. Study design

A cross-sectional epidemiological study was conducted from November 2021 to April 2022 to assess the prevalence of bovine mastitis and identify its major predisposing factors in dairy cattle. The study employed a quantitative research approach using a structured questionnaire, clinical examination, and laboratory analysis to collect data from selected dairy farms. A multivariable logistic regression model was applied to determine associations between potential risk factors and mastitis occurrence. The scope of the study was limited to dairy farms within the study area, representing both smallholder and semi-intensive production systems.

2. 5. Study methodology

A combination of semi-structured questionnaires, direct observations, and the California Mastitis Test (CMT) were used to collect key data for this study. Clinical mastitis in lactating cows was evaluated through direct inspection, while subclinical mastitis was identified with CMT screening, following the method described by Quinn et al. (1999). A total of 510 lactating cows and 2,040 teat quarters were examined for both clinical and subclinical mastitis. Additionally, farm attendants completed a semi-structured questionnaire to identify intrinsic and extrinsic factors contributing to mastitis.

2. 6. Data Collection

2. 6. 1. Questionnaire Survey

A semi-structured questionnaire was designed and pretested to collect data on the impact of selected risk factors associated with mastitis. The information gathered included breed, age, lactation stage, parity, milk production, body condition, and previous history of mastitis, presence of lesions on the udder or teat skin, and milking practices. Additionally, udder and milk abnormalities such as injuries, blindness, swelling, milk clots, and abnormal secretions like blood or pus were documented. Lactation stages were classified as early (1–3 months), mid (4–6 months), and late (7 months and above), while age groups were categorized as young (3–6 years) and adult (6–8 years). Parity was grouped into few (1–3 calves), moderate (4–6 calves), and many (7 or more calves)(Abera, *et al*.,2010). Milk yield was categorized as low (7 liters), medium (8–15 liters), and high (greater than 15 liters) (Ortegon, 2013; Mureithi & Njuguna, 2016; Yilma *et al*., 2017).

2. 6. 2. Physical Examination of Udder and Milk

The udders underwent a thorough examination and palpation to identify any indications of fibrosis, inflammation, tissue atrophy, damage, or enlargement of the per-mammary lymph nodes. The mammary quarters were evaluated for abnormalities in size and consistency, including asymmetry, swelling, stiffness, discomfort, and blindness. Additionally, milk secretion from each teat quarter was assessed for viscosity and visual characteristics to detect clots, flakes, blood, or watery discharges (Biffa *et al*., 2005).

2. 6. 3. California mastitis test (CMT)

The California Mastitis Test was performed by the method outlined by Quinn *et al.* (1999). The cow's udder was first cleansed using antiseptics and water; then dried with a clean towel. The initial few drops of milk were discarded from each udder quarter before collecting samples. Subsequently, two milliliters of milk were placed into each of the four shallow cups on the CMT paddle, followed by the addition of an equal volume of CMT reagent. The mixture in each cup was gently stirred in a horizontal plane for 15–20 seconds. The reaction was then graded as 0 and traced for negative results, while positive results were classified as +1, +2, or +3.

2. 6. 4. Milk sample collection, handling and transportation

Milk samples were collected following standard milk-sampling procedures from all teats affected by clinical mastitis (CM) and subclinical mastitis (SCM) (NMC, 1990). Before sample collection, the udder, especially the teats, was thoroughly cleaned and dried. Dust, bedding particles, and other debris were removed by brushing with a dry towel, after which the teats were washed with tap water and dried. Next, the teats were disinfected using cotton swabs soaked in 70% alcohol (NMC, 1990). To avoid recontamination during the alcohol scrubbing process, the far-side teats were cleaned first, followed by the near-side ones. During sample collection, the near teats were sampled first, followed by the far ones to minimize contamination of the teat ends. After discarding the first three milking squirts, ten milliliters of milk were collected into sterile test tubes. The samples were then labeled with a temporary identification number assigned to each cow, stored in an icebox, and transported to the microbiology laboratory at Addis Ababa

University College of Veterinary Medicine (NMC, 1990).

2. 6. 5. Bacteriological isolation and identification of causative agents of mastitis

Bacteriological examination of milk samples from both clinical and subclinical quarters was conducted following the procedures outlined by Quinn *et al*. (2002). A loopful of milk from each infected quarter was separately inoculated onto MacConkey agar and blood agar base enriched with 7% defibrinated bovine blood. The inoculated plates were incubated aerobically at 37°C for 24 to 48 hours, after they were inspected for bacterial growth, colony morphology, and hemolytic characteristics on blood agar. Suspected colonies were subsequently subcultured onto fresh plates for further analysis. Primary bacterial cultures were identified by colony morphology, and hemolytic characteristics, a Gram stain to determine Gram negative/positive status like catalase testing, and oxidative-fermentative (O-F) to further differentiate and identify the specific bacteria. Key bacterial pathogens, including *Staphylococcus species, Streptococcus species*, and *Escherichia coli (E. coli),* were targeted as they are the principal causative agents of bovine mastitis. Suspected colonies were examined both morphologically and microscopically.

Staphylococci were identified through catalase testing, growth characteristics on Mannitol salt agar and purple agar, as well as tube coagulase testing. Streptococci were characterized using growth patterns on Edwards media, catalase testing, and esculin hydrolysis. Gram-negative isolates cultured on MacConkey agar were identified based on their growth characteristics on MacConkey agar, oxidase reaction, catalase testing, triple sugar iron (TSI) agar results, and the IMViC tests (indole, methyl red, Voges-Proskauer, and citrate) (Quinn *et al*., 2002).

2. 7. Data analysis and management

The collected data during the study period were entered into a Microsoft Excel 2016 spreadsheet for organization and processing. Descriptive statistics were applied to determine the prevalence of mastitis, while STATA 15 statistical software was used for analysis. A chi-square test was conducted to evaluate the relationship between various risk factors and mastitis occurrence. Additionally, multivariable logistic regression was performed to assess associations between mastitis and factors such as breed, age, teat lesions, lactation stage, milk yield, herd size, farm hygiene, farm type, farm location, and udder washing. Statistical significance was determined for factors with a p-value of less than 0.05.

3. RESULTS

A total of 510 lactating cows comprising 466 cross-breeds, 24 local indigenous breeds, and 20 Jersey breeds were assessed for mastitis through physical examination and the California Mastitis Test (CMT). Among them, 368 cows (72.2%) tested positive for mastitis, with 88 (17.3%) cases classified as clinical mastitis and 280 (54.9%) as sub-clinical mastitis. Clinical mastitis was characterized by the presence of clots, flakes, bloody, or watery discharges in the milk. Out of 2,040 quarters examined, 55 (2.7%) were found to have blind teats. Among the remaining 1,985 functional quarters, 316 (15.9%) exhibited signs of clinical mastitis (Table 1).

Table 1.Prevalence of mastitis in lactating cow

Disease status	Number of animals examined	Number of positive (%)	RH	RF	LH	LF	Total quarter prevalence
Subclinical mastitis	510	280(54.90%)	194	211	202	210	817(41.2%)
Clinical	510	88(17.3%)	81	80	80	75	316(15.9%)
Blind teats	-	-	18	15	10	12	55(2.70%)
Negative (0)	510	142(27.8%)	217	204	218	213	852(41.76%)
Total	510	368(72.2%)	510	510	510	510	2040(100)

RF= Right Front; RH= Right Hind; LF= Left Front; LH= Left Hind

3. 1. Association of Risk Factors with the Prevalence of Clinical Mastitis

A significant association was found between cow age and clinical mastitis ($\chi^2 = 12.25$, P = 0.000). Adult cows had 2.38 times higher odds of testing positive for mastitis via the CMT compared to younger cows (OR = 2.38; 95% CI: 1.45–3.92). Breed was also significantly correlated with mastitis prevalence. Additionally, cows with a previous history of clinical mastitis were 5.26 times more likely to test positive again (OR = 5.26; 95% CI: 3.22–8.59). Parity showed a significant association ($P < 0.05$), with cows having four or more calvings exhibiting a higher infection rate than those with fewer calvings ($\chi^2 = 4.42$, P = 0.035). Regarding teat lesions, mastitis positivity was highest in cows with teat injuries ($\chi^2 = 78.12$, P = 0.000).

Mastitis prevalence was also higher in high milk-producing cows, which were 4.21 times more likely to be mastitis-positive compared to low- or medium-producing cows (OR = 4.21; 95% CI: 2.10–8.41). Larger herd sizes (≥21 cows) were associated with increased infection rates, with cows in these herds having 3.31 times higher odds of mastitis than those in small or medium herds. Furthermore, cows with washed udders had 4.8 times higher odds of CMT positivity compared to unwashed udders

(OR = 4.76; 95% CI: 1.7–13.4). The detailed associations between various host and environmental factors and mastitis are presented in Tables 2 and 3

Table 2. Association of risk factors for the occurrence of clinical mastitis

Host Factors	Categories	Animal examined	Number Positive (%)	OR[95%CI]	χ2	P-value
Age	Young(≤6)	237	26(11.0)			
	Adult(≥ 7)	273	62(22.7)	2.38[1.45-3.92]	12.25	0.000
Breeds	Local	24	0(0.0)			
	Jersey	20	0(0.0)			
	Cross	466	88 (18.9)	1.12[0.51, 2.45]	10.04	0.007
Mastitis history	No	395	43(10.9)			
	Yes	115	45(39.1)	5.26[3.22-8.59]	49.77	0.000
Number of parity	1-3 calves	355	53(14.9)			
	≥4 calves	155	35(22.6)	1.66[1.03-2.68]	4.42	0.035
Teat lesion	Absent	449	53(11.8)			
	Present	61	35(57.4)	10.49[5.83-18.88]	78.12	0.000
Milk Yield	Low	173	12(6.9)			
	Medium	182	39(21.4)	3.66[1.84-7.26]		
	High	155	37(23.9)	4.21[2.10-8.41]	19.87	0.000
Lactation stage	Early	132	26(19.7)			
	Mid	121	28(23.1)	1.23[0.67-2.24]		
	Late	257	38(13.2)	0.62[0.35-1.09]	6.40	0.057
Body condition	Poor	39	9(23.1)			
	Good	294	58(19.7)	0.82[0.37-1.82]		
	Moderate	177	21(11.9)	0.449[0.187-1.07]	5.79	0.072
Herd size	Small	243	23(9.5)			
	Medium	127	29(22.8)	2.83[1.56-5.14]		
	Large	140	36(25.7)	3.31[1.87-5.87]	21.38	0.000

CI=Confidence interval, OR= Odds ratio, χ2=Chi-square. p<0.05=Significant

Table 3. Association between clinical mastitis and environmental factors

Host Factors	Categories	Animal examined	Number Positives (%)	OR[95%CI]	χ2	P-value
Udder Washing	No	82	4(4.9)			
	Yes	428	84(19.6)	4.8[1.7-13.4]	10.48	0.001
Towel usage	No	297	47(15.8)			
	Yes	213	41(19.2)	0.79[0.5-1.25]	1.02	0.31
Udder drying	No	238	36(15.1)			
	Yes	272	52(19.1)	0.75[0.47-1.20]	1.42	0.23
Milking mastitis cow at the end	No	194	21(10.8)			
	Yes	316	67(21.2)	0.65[0.40-1.06]	2.97	0.085
Type of farm	Extensive	80	9(11.2)			
	Semi-intensive	95	29(30.5%)	2.5[1.47-4.26]		
	Intensive	335	50(14.9%)	0.72[0.34-1.54]	15.01	0.001

CI=Confidence interval, OR= Odds ratio, χ2=Chi-square. $p<0.05$=Significant

3. 2. Associations of Risk Factors with Subclinical Mastitis Prevalence

As shown in Table 4, multiple factors, including age, breed, milk yield, lactation stage, teat lesions, and herd size, were significantly ($P < 0.05$) associated with subclinical mastitis prevalence (SCM). Age of the cow showed a significant association ($P \leq 0.05$), with adult cows having a higher SCM prevalence (59.0%) compared to younger cows (50.2%). Young cows were 1.43 times less likely to test positive for SCM (OR: 1.43; 95% CI: 1.02–2.05). Breed also influenced SCM prevalence, with the highest rates observed in Holstein Friesian crosses (55.8%), followed by Jersey (60%) and Indigenous Zebu breeds (33.3%). Milk yield was another significant factor, with cows producing >15 liters/day showing the highest SCM prevalence (65.8%), compared to 50.0% in those yielding 7–15 liters and 50.3% in those producing <7 liters. High-yielding cows (>15 liters) had twice the odds of SCM compared to low-yielding cows (<7 liters) (OR = 1.90; 95% CI: 1.22–2.97). Lactation stage was strongly associated ($P = 0.000$) with SCM prevalence, which was highest in early lactation (67.4%), followed by mid- (54.5%) and late-lactation stages (48.6%). Body condition showed a trend, with poor-condition cows having the highest SCM prevalence (64.1%), followed by moderate (53.7%) and good-condition cows (54.4%), though these differences were not statistically significant (P > 0.05).

Table 4. Association between the occurrence of subclinical mastitis and risk factors

Host Factors	Classes	Animal examined	Mastitis Positive (%)	95%CI	OR	P-value
Breed Types	Local cows	24	8(33.33)			
	Cross breeds	466	260(55.8)	1.09-6.01	2.59	0.037
	Jersey	20	12(60.0)	0.87-10.30	3.0	0.081
Age Category	Young (≤6)	237	119(50.2)			
	Adult (≥7)	273	161(59.0)	1.0-2.02	1.43	0.048
Number of Animals	1-3 calves	355	187(52.7)			
	≥4 calves	155	93(60.0)	0.92-2.1.98	1.35	0.127
Lactation stage	Late	257	125(48.6)			
	Mid	121	66(54.5)	0.82-1.955	1.267	0.284
	Early	132	89(67.4)	1.41-3.389	2.19	0.000
Mastitis history	Yes	115	57(49.6)			
	No	395	223(56.5)	0.87-2.0	1.32	0.192
Teat lesion	Present	61	21(34.4)			
	Absent	449	260(57.7)	1.9-5.20	3.14	0.000
Milk Yield	Low	173	87(50.3)			
	Medium	182	91(50.0)	0.65-1.50	0.989	0.957
	High	155	102(65.8)	1.22-2.97	1.90	0.005
Body condition	Poor	39	25(64.1)			
	Moderate	177	95(53.7)	0.32-1.33	0.65	0.237
	Good	294	160(54.4)	0.33-1.34	0.67	0.255
Herd size	Small	243	121(49.8)			
	Medium	127	83(65.4)	1.22-2.96	1.90	0.005
	Large	140	76(54.3)	0.789-1.82	1.20	0.487

OR= Odds ratio, P<0.05= statistically significant

3. 3. Environmental Risk Factors for Subclinical Mastitis (SCM)

This study identified several environmental factors significantly influencing SCM prevalence: Pre-milking udder washing was associated with significantly higher SCM prevalence (59.6%) compared to unwashed udders (31.7%). The odds of SCM were 3.14 times greater in washed udders (95% CI: 1.9-5.2). Towel drying after washing showed marginally higher SCM rates (55.9%) compared to air-drying (54.2%). Poor farm hygiene was associated with a 1.67-fold increased risk of SCM compared to well-maintained farms (OR = 1.67; 95% CI: 1.18–2.39).

Table 5. Association between subclinical mastitis and environmental factors

Host Factors		**Animal examined**	**Mastitis Positive (%)**	**95%CI**	**OR**	**P-value**
Farm Location	Urban	247	155(62.8)	0.38-0.77	0.54	0.001
	Peri-urban	263	125(47.5)			
Type of Farm	Extensive	80	30(37.5)			
	Semi-intensive	95	40(42.1)	0.66-2.23	1.2	0.54
	Intensive	335	210(62.7)	1.7-4.6	2.8	0.000
Udder Washing	No	82	26(31.7)			
	Yes	428	254(59.3)	1.9-5.2	3.14	0.000
Udder Drying	Yes	272	148(54.4)			
	No	238	132(55.5)	0.74-1.48	0.94	0.710
Towel Usage	Yes	213	119(55.9)			
	No	297	161(54.2)	0.66-1.33	0.94	0.710
Milking a Mastitis cow last	Yes	316	154(48.7)			
	No	194	126(64.9)	0.35-0.74	0.51	0.000
Farm Hygiene	Good	276	136(49.3)			
	Bad	234	144(61.5)	1.18-2.39	1.67	0.004

OR= Odds ratio, P<0.05= statistically significant, CI=Confidence interval

3. 4. Analyses of multivariable logistic regression for subclinical mastitis

As presented in Table 6, all risk factors that showed an association with sub-clinical mastitis at a significance level of $p \leq 0.25$ in the univariable analysis were included in the initial multivariable logistic regression models. The comprehensive model incorporated variables such as breed, age, teat lesions, lactation stage, milk yield, herd size, farm hygiene, farm type, farm location, and udder washing practices. All variables with $p < 0.25$ from the univariable analysis were further assessed for multicollinearity. The final analysis identified several factors significantly associated with the occurrence of sub-clinical mastitis ($p < 0.05$), including: Breed and age of cows, Presence of teat lesions, Lactation stage, Farm type and hygiene practices, Udder washing protocols, and Order of milking (mastitic cows milked last).

Table 6. Multivariable logistic regression analyses identifying the association of risk of subclinical

Host Factors	Category	OR	95% CI for OR	P-value
Breed	Local Zebu	Ref		
	Cross breeds	3.11	1.19-8.13	0.021
	Jersey	3.6	0.91-14.19	0.068
Age	Young(≤6)	Ref		
	Adult(≥7)	2.06	1.34-3.18	0.001
Type of farm	Intensive	Ref		
	Semi-intensive	0.40	0.23-0.70	0.001
	Extensive	0.41	0.19-0.89	0.024
Herd size	<10			
	10-20	1.66	0.95-2.91	0.078
	≥21	1.31	0.69-2.51	0.410
Stage of lactation	Early			
	Mid	0.50	0.27-0.91	0.023
	Late	0.37	0.21-0.64	<0.001
Farm hygiene	Good	Ref		
	Bad	1.86	1.19-2.90	0.006
Teat lesion	Present	Ref		
	Absent	3.77	1.98-7.18	<0.001
Udder washing	No	Ref		
	Yes	0.32	0.18-0.58	<0.001
Milking a mastitic cow last	Yes	Ref		
	No	2.0	1.18-3.40	0.010

OR= Odds ratio, P<0.05= statistically significant, Ref=reference

3. 5. Bacterial Isolation

As summarized in Table 7, all milk samples collected from cows with clinical mastitis and CMT-positive results tested positive for various bacterial species. Out of 368 CMT-positive cows, 144 milk samples were cultured for bacterial isolation. Both contagious pathogens (e.g., *Staphylococcus* spp. and *Streptococcus* spp.) and environmental pathogens (e.g., *Escherichia coli*) were identified. *Staphylococcus* spp. was the most frequently isolated pathogens, with *Staphylococcus* aureus accounting for the largest proportion (40.3%). Overall, the most commonly isolated bacteria were: *Staphylococcus* spp. (57.7%), *Streptococcus* spp. (24.3%) and *Escherichia coli* (8.3%). A total of 83 samples were positive for *Staphylococcus* spp. The detailed distribution of isolated bacterial species is presented in Table 7.

Table 7. Frequency distribution of isolated bacteria from mastitis dairy cows

Bacterial Isolates	**Frequency**	**Prevalence (%)**
Staphylococcus aureus	58	40.3%
Staphylococcus intermedines	2	1.4
Staphylococcus hycus	5	3.5
Coagulase negative staphylococcus (CNS)	18	12.5
Streptococcus species	35	24.3
Escherichia coli	12	8.3
Mixed growth	14	9.7
Total	144	100

4. DISCUSSION

The study found an overall mastitis prevalence of 72.2% (95% CI: 68.5–75.7), with subclinical mastitis representing the majority of cases (54.9%), compared to clinical mastitis (17.3%). These results are consistent with previous findings in Ethiopia, including a 71.0% prevalence in Holeta Town (Mekibib *et al.,* 2010) and 74.7% in Addis Ababa and surrounding areas (Zeryehun *et al*., 2013). However, the prevalence observed in this study is lower than reports from the North Shewa Zone, where Dabash *et al*. (2014) and Kifle & Tadele (2000) documented rates of 88.9% and 89.5%, respectively. In contrast, lower prevalence rates have been reported in Borana (59.1%) (Adane *et al*., 2012), Batu and its surroundings (56.5%) (Bedacha & Mengistu, 2011) and in the pastoral areas of Borana zones (50.03%) (Dinaol *et al*., 2016).

The study found 54.9% subclinical mastitis prevalence at the cow level, demonstrating substantial economic impact on the dairy sector. This prevalence imposes high loss on the dairy production. This result is consistent with previous research, such as Bikila and Soressa (2022), who reported a prevalence of 55.7% in and around Holeta Town, Oromia, Ethiopia, as well as findings by Zeryehun *et al*. (2013), Abera *et al.* (2013), and Abebe *et al*. (2016), who documented prevalence rates of 55.1%, 54.4%, and 59.2%, respectively, in dairy farms located in Addis Ababa, Adama Town, and the Hawassa milk shed. However, the prevalence reported in the current study is lower than those found in earlier studies, including 85.4% by Tadesse *et al*. (2014) in Addis Ababa, 62% by Sefinew (2018) in North-Western Ethiopia, and 73.3% by Ararsa *et al*. (2013) in Holeta, Central Ethiopia. The observed variations in prevalence likely reflect differences in environmental conditions and farm management practices. This study supports the widely accepted view that subclinical mastitis is more prevalent than clinical mastitis, aligning with the findings of Radostits *et al*. (2007), who reported that subclinical cases occur three to four times more frequently than clinical ones.

From a public health perspective, both clinical and subclinical mastitis have notable implications. Clinical mastitis can lead to visible abnormalities in milk, making it unfit for human consumption and posing risks if contaminated milk enters the food chain. In contrast, subclinical mastitis, though not outwardly detectable, is more insidious as affected cows continue to produce milk that may contain pathogenic bacteria and antibiotic residues, thereby contributing to foodborne infections and antimicrobial resistance (AMR) development among consumers. The unnoticed nature of subclinical infections makes them a hidden threat to public health, as contaminated raw or inadequately pasteurized milk can serve as a reservoir for zoonotic pathogens such as Staphylococcus aureus, E. coli, and Streptococcus species. This study supports the widely accepted view that subclinical mastitis is more prevalent than clinical mastitis, aligning with Radostits *et al.* (2007), who reported that subclinical cases occur three to four times more frequently than clinical ones.

The 17.3% prevalence of clinical mastitis in this study aligns with other Ethiopian dairy farms studies, such as 18.35% testified by Abebe and Bakala (2022) near Holeta Town, 19.6% by Zeryehun *et al*. (2013) in Addis Ababa, and 16.67% by Tuke *et al*. (2017) at Allage ATVET College Dairy Farm in Southern Ethiopia. However, this rate is higher than 4.4%, 5.3%, and 7.8%, reported by Bedacha and Mengistu (2011), and Ararsa *et al.* (2013), who found clinical mastitis prevalence rates of respectively, in dairy farms located in dairy farms around Gondar, Batu and the Holeta Agricultural Research Center. In line with previous studies by Biffa *et al*. (2005), Sori *et al*. (2005), Almaw *et al*. (2008), Lakew *et al*. (2009), and Haftu *et al.* (2012), the current findings reaffirm that clinical mastitis occurs less frequently than subclinical mastitis. This disparity is likely due to the lack of visible symptoms in subclinical cases, leading to under-diagnosis and minimal intervention. Farmers, particularly smallholders, often lack awareness about the hidden economic losses associated with subclinical mastitis. As noted by Almaw *et al.* (2008), clinical mastitis tends to receive more attention in Ethiopia, while subclinical cases remain largely neglected.

The overall prevalence of clinical and subclinical mastitis at the quarter level was 15.9% and 41.2%, respectively. The quarter prevalence of subclinical mastitis (41.2%) observed in this study is comparable to the findings of Zeryehun and Abera (2017) and Fesseha *et al*. (2021), who reported 41.4% and 36.9% in the eastern Harrarghe Zone and Modjo Town and Suburbs, Central Oromia, Ethiopia, respectively. However, this finding is lower than the reports by Fesseha *et al*. (2021) (3.4%) and Kebebew and Jorga (2016) (5.5%) regarding blind quarters in their studies. The most frequent risk factors contributing to quarter blindness include inadequate monitoring of clinical and chronic mastitis, failure to detect and treat subclinical infections, and persistent microbial challenges to the mammary glands. Over time, this gradual and often unnoticed degeneration of mammary tissue can ultimately lead to non-functional or blind quarters.

The present study found a significant association between cow age and the prevalence of clinical mastitis ($\chi^2 = 12.25$, $p = 0.000$). Adult cows over 7 years of age were found to have 2.38 times higher odds of testing positive on the California Mastitis Test (CMT) compared to younger cows. However, this result contrasts with the findings of Kerro and Tareke (2003), who reported that the risk of both clinical and subclinical mastitis increases progressively with age. As noted by Radostits *et al.* (2007), older cows typically have larger teat canals and more relaxed sphincter muscles, which can facilitate pathogen entry into the udder, thereby increasing susceptibility to infection. The higher prevalence of mastitis with increasing parity observed in the current study is consistent with earlier findings by Dabele *et al*. (2021) and Nebyou and Birtukan (2023). This trend may be attributed to the fact that primiparous cows generally possess a stronger immune response compared to their multiparous counterparts (Erskine, 2001). In multiparous cows, the accumulated exposure to pathogens and longer duration of previous infections likely contribute to a greater risk of mastitis over time (Radostits *et al*., 2007).

The study reveals the prevalence of mastitis to be significantly affected ($p < 0.05$) by age. In the present study, age was a factor that increased the likelihood of detection, with adult cows being 1.43 times more likely to have mastitis compared to young cows. This aligns with previous findings by Moges et al. (2011), Zeryehun et al. (2013), Zenebe et al. (2014), Abebe et al. (2016), and Zeryehun and Abera (2017). The higher prevalence in older cows is due to their larger teats and more relaxed sphincter muscles, which increase the accessibility of infectious agents in the cows' udder (Radostits *et al*., 2007).

Jersey-bred cows were affected at a higher rate (60.0%) than HF cross (55.8%) and indigenous zebu (33.33%). This result is by result of Tesfanesh *et al*. (2018), Moges *et al. (*2012), and others. However, the difference in this study was not statistically insignificant (p 0.089). A high prevalence of mastitis was observed in cows with a previous history mastitis disease, a finding supported by the findings of Tesfanesh *et al*., (2018). Absence of a dry cow therapy regime could be the major factor, contributing to the high prevalence in previously treated animals and to the development of bacterial resistance to treatment due to lack of observance of the full course of antibiotic treatment or the habit of changing therapy in an inappropriate manner.

Similarly, the risk factors considered for this study were teat lesion, stages of lactation, and milk yield. The occurrence of mastitis based on these risk factors, like teat lesion, stages of lactation, and milk yield, showed a statistically significant effect on the occurrence of subclinical mastitis. This was also reported by several investigators to have an association with the occurrence of mastitis (Mekibib, 2010; Nibret, 2012; Hagos, 2015).

Multivariable logistic regression analysis identified several significant risk factors ($p < 0.05$) associated with subclinical mastitis. Specifically, breed, age, farm type, teat lesions, udder washing practices, lactation stage, and the order of milking mastitic mastitis cows were all significantly linked to the occurrence of subclinical mastitis in lactating dairy cows. The odds of detecting a cow with a positive California Mastitis Test (CMT) result were 2.06 times higher in cows aged 7 years or older compared to younger cows. This finding aligns with previous studies by Moges *et al*. (2011), Zeryehun *et al.* (2013), and Zenebe *et al.* (2014). Additionally, cows from herds where mastitic cows were not milked last had a significantly higher likelihood of developing mastitis, consistent with reports from other regions of the country (Abebe *et al*., 2016; Belayneh *et al*., 2013). Failure to milk mastitic cows last promotes the transmission of mastitis-causing pathogens via the milker's hands, thereby increasing the risk of contagious mastitis (FAO, 2014). Interestingly, the study also observed a high prevalence of mastitis among cows without visible teat or udder lesions. This may be due to prior treatments that removed visible lesions. Farnsworth (1995), who found a positive association between teat-end condition and subclinical mastitis, supports this observation.

Farm hygiene was also significantly associated with mastitis prevalence. The odds of mastitis were 1.86 times higher in farms with poor hygiene. This finding is consistent with previous studies (Abrahmsén *et al*., 2014; Iraguha *et al*., 2015; Mureithi and Njuguna, 2016), which emphasized that dirty floors can serve as a reservoir for mastitis-causing organisms, particularly environmental pathogens. Lack of a proper waste drainage system and the accumulation of manure and urine may facilitate pathogen entry into the udder through the teat orifice.

Cows in early lactation were susceptible to mastitis compared to those in mid and late lactation stages. Early lactation cows are at lower risk for mastitis; studies by Elbably *et al.* (2013), Zeryehun *et al.* (2013), Zenebe *et al.* (2014), and Iraguha *et al*. (2015) have reported similar trends. This could be due to the delayed infiltration of neutrophils into the mammary gland post-calving (Radostits *et al.,* 2007) and the increased oxidative stress coupled with decreased antioxidant defense mechanisms during early lactation (Sharma *et al*., 2011).

Moreover, the absence of dry cow therapy may contribute to the increased mastitis prevalence during early lactation. However, Belayneh *et al*. (2013) reported contrasting findings, observing a higher mastitis prevalence in the late lactation stage.

The current study also highlighted a high prevalence of bovine mastitis, with Staphylococcus, Streptococcus, and Escherichia coli identified as the predominant bacterial isolates. This concurs with previous findings by Abera *et al*. (2012) and Sefinew (2018). Similar reports from other parts of the country indicated Staphylococcus aureus as the most common pathogen (Abera *et al*., 2013; Belayneh *et al*., 2013; Gebremichael *et al*., 2013; Zeryehun *et al*., 2013; Duguma *et al*., 2014). The FAO (2014) also reported S. aureus as a major etiological agent of mastitis in both African and Asian dairy cattle populations.
Several management factors may contribute to the high prevalence of S. aureus, including the failure to cull chronically infected cows, lack of post-milking teat disinfection, absence of dry cow therapy, and the use of a common bucket for udder washing. These pathogens are typically located on the teat or udder skin of infected animals and are primarily transmitted during milking (Abebe *et al.,* 2016). Although all assessed farms reported washing hands before milking, this practice was only performed before milking the first cow, increasing the likelihood of cross-contamination.

Radostits *et al*. (2000) noted that S. aureus is well-adapted to the udder environment, often establishing chronic subclinical infections that are shed in milk and easily transmitted during milking.

5. CONCLUSION AND RECOMMENDATIONS

Improving the existing gaps through structured interventions and active farmer participation is essential for reducing mastitis prevalence, improving dairy productivity, and protecting both livelihoods and public health. Staphylococcus spp. emerged as the most prevalent causative agents, followed by Streptococcus spp. Unlike clinical mastitis, subclinical infections often remain undetected due to the absence of visible symptoms, resulting in insufficient treatment and inadequate control measures. None of the surveyed farms practiced routine mastitis prevention strategies such as post-milking teat disinfection, wearing gloves during milking, administering dry cow therapy during the non-lactating period, culling chronically infected cows, or conducting regular screening such as the California Mastitis Test (CMT) for subclinical cases. Additionally, poor barn sanitation, inadequate bedding, and lack of udder hygiene during milking further increased the risk of environmental mastitis. The high prevalence of mastitis significantly compromises milk yield and quality, leading to substantial economic losses and posing serious public health concerns, especially when contaminated milk enters the food chain. To address these challenges, a comprehensive mastitis control program is recommended, incorporating the following components:

Subclinical mastitis should be monitored through regular fortnightly screening using diagnostic methods such as the California Mastitis Test (CMT).

Strict adherence to pre- and post-milking udder hygiene practices should be enforced, including the use of antiseptic teat dips and mandatory glove use by milkers.

Dry cow therapy should be administered during the non-lactating period to prevent new intramammary infections following calving.

Sanitary housing conditions must be maintained by providing clean bedding, efficient waste disposal, and overall barn hygiene.

Clinical mastitis cases should be treated promptly, and cows with chronic infections should be culled to prevent disease spread. Dairy farmers should receive training on mastitis prevention strategies, the economic impact of the disease, and the potential zoonotic risks.

Further research should be conducted to characterize mastitis-causing pathogens, with a particular focus on antimicrobial resistance and the public health risks associated with milk-borne infections.

Acknowledgments

The authors would like to acknowledge Addis Ababa University, Research and Technology Transfer, and Debre Berhan University for their financial support. We also extend our sincere thanks to the staff of the Debre Berhan, BosonaWorana and AngolelaenaTera district Livestock and Fishery Development offices, development agents who assisted in data collection and livestock owners for their kind cooperation.

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