Prevalence of bovine mastitis, risk factors, changes in milk composition and bacterial isolation in and around Modjo town, central Ethiopia

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Abstract

A cross-sectional study was conducted on 316 lactating dairy cows from November 2021 to May 2022 in and around Modjo. The main objectives of this study include estimating the prevalence and isolation of aerobic bacterial pathogens and observing compositional changes in mastitic milk. To this end, mastitis was diagnosed both clinically and using California mastitis test (CMT). Isolation of major aerobic pathogens and alterations in milk composition were determined using their respective standard techniques. Accordingly, the overall prevalence of mastitis observed in the study was 72.8%, of which 9.5% was clinical and the rest 63.3% subclinical mastitis. Quarter level prevalence was 45.2%. Among the potential risk factors considered, the presence of teat/udder injury, poor udder and farm hygiene showed higher prevalence of mastitis ($p < 0.05$) compared to the corresponding categories. The most predominant bacterial pathogen isolated was *Staphylococcus aureus* (11.25%), followed by *E. coli* (10%), *Micrococcus* species (10%) and *Pseudomonas aeruginosa* (10%). Protein, solid non-fat (SNF), pH and freezing point measurements showed statistically significant difference ($p < 0.05$) between milk from mastitic and non-mastitic udders. However, fat and density were not statistically different ($p > 0.05$). In conclusion, mastitis was highly prevalent and clinically important disease of dairy cows in the study area and hence deserves attention to minimize its impact. Cows' udder and farm should be kept clean as much as possible to reduce the prevalence of mastitis. Some of these bacterial isolates can be the real causes of mastitis. However, studies should be conducted to establish causal relationship.

Keywords: Bacteria; Bovine mastitis; Ethiopia; Milk; Modjo; Prevalence.

Introduction

Ethiopia is believed to have the largest livestock population in Africa. The total cattle population of the country is estimated at 61.5 million. Of this total cattle population, female cattle constitute about 55.7 percent, and the remaining 44.3 percent are male cattle (CSA, 2019).

Even though cow milk is a vital source of nutrition for many people in Ethiopia, the country has long failed to satisfy the demand for milk and milk products through domestic production. Multiple factors contribute to this, one of the major reasons being the high prevalence of mastitis in dairy cows in the country (Fekadu, 1995).

Mastitis, one of the most economically important diseases for the dairy industry worldwide, continues to cause serious problems through reduced milk production (Korhonen and Kaartinen, 1995; Bradley, 2002). It can also pose a risk to human health (Al-Majali *et al.,* 2008), as bacterial contamination of milk from the affected cows may render it unsafe for human consumption (Quinn *et al.,* 1999).

Mastitis (Greek, "mastos" = breast + "itis" = inflammation), defined as inflammation of the parenchyma of the mammary glands, is a complex multi-etiological disease characterized by physical, chemical and usually bacteriological changes in milk, and pathological changes in the mammary gland tissues (Radostits *et al.,* 2007).

In dairy cattle, mastitis can have an infectious or non-infectious etiology, although the vast majority is of bacterial origin (Anon, 2001). Mastitis continues to be the most economically important disease of the dairy industry, accounting for about 38% of the total direct losses (Albenzio *et al.,* 2002).

There are two main types of bacteria that cause mastitis: contagious and environmental. Contagious bacteria, such as *Staphylococcus aureus* and *Streptococcus agalactiae*, spread easily between cows. Environmental bacteria, such as coliforms and some streptococci, live in the milking environment and can infect cows during milking. The risk of mastitis is likely to increase with poor milking sanitation, low barn sanitation, lack of teat dipping after milking, use of lubricants during milking, and lack of treatment of existing infection in milking cows of various age groups (Radostits *et al*., 2007).

Mastitis can be classified as clinical or subclinical. Clinical mastitis cases are characterized by the presence of one or more of the following signs such as abnormal milk, udder swelling and systemic signs including elevated temperature, lethargy and anorexia. In subclinical mastitis there are no visible changes in the milk or the udder, but milk production decreases, bacteria are present in the milk and composition of the milk is altered. For every case of clinical mastitis there are 20-40 times as many cases of subclinical mastitis (Eriskine, 2001). Subclinical mastitis in the mammary gland is detectable only by determining high somatic cell count (SCC) in milk or by bacterial culture (Quinn *et al.,* 1994).

Even though subclinical mastitis isn't as overt as clinical mastitis, it causes the greatest financial losses to the dairy industry. It is estimated to cause about 70% of the total losses. These losses, however, are difficult for producers to appreciate since they are associated with decreased milk production caused by the effects of chronic inflammation of the mammary gland without observable clinical signs (Radostits *et al.,* 2007).

One consequence of mastitis in dairy cows is a change in the composition of milk (Gianneechini *et al.,* 2002). An increase in pathogenic bacteria in milk is accompanied by an increase in somatic cell count and changes in milk physicochemical properties (Santos *et al.,* 2004; Rerk *et al.*, 2008).

Studies conducted in different parts of the country have shown that mastitis is a widespread problem in Ethiopia, with prevalence ranging from 6% to 74.7% (Biffa *et al*., 2005; Sori *et al.,* 2005; Mekbib *et al.,* 2010; Zeryehun *et al.,* 2013; Belachew, 2016; Abebe *et al.,* 2017; Tesfaye and Abera, 2018). However, there is a need for more information on the occurrence of bovine mastitis in and around Modjo town. Moreover, while these earlier studies explored the prevalence, the associated risk factors, and the bacteria involved in mastitis, there is a need for more information on the effect of mastitis on milk composition in the study area.

Therefore, the objectives of this study were to estimate the prevalence of mastitis, identify associated risk factors, isolate aerobic bacteria from mastitic milk, and study changes in milk composition in cows with mastitis in the study area.

Materials and methods

Study area description

The study was conducted in Modjo town from November 2021 to May 2022. Modjo is the administrative center of Lume wereda, located in the East Shewa Zone of the Oromia Region, Ethiopia. It is located at 73 km south east of Addis Ababa at an altitude of 1,777m above sea level. It is located at 8.36°N latitude and 39.7°E longitude. The monthly mean minimum and maximum temperature for Modjo town ranges from 8.5°C-13.5 °C to 25.6-30.8 °C, respectively (CSA, 2012).

Study animals

The study animals were Holstein-Zebu crossbred milking cows in selected small -and large-scale dairy farms and villages in Modjo town and the surroundings. The study animals were classified as young adults (3–6 years), and adults (≥7 years) based on their dentition (Wakeman and Pace, 1983). The herd size was categorized as few (< 10) and many (≥ 10). The number of parities was classified as few (1-3) and many (≥ 4). The stage of lactation was categorized as early (1-3 months), mid (4-6 months) and late (≥ 7 months).

Study design and sampling strategy

A cross-sectional study was conducted from November 2021 to May 2022. Lume Wereda agriculture office provided a list of dairy farms.Then, study farms and households were selected by simple random sampling, and all lactating cows in the selected farms were included in the study. This was done until the desired sample size was met.

Sample size determination

The desired sample size for the study was calculated using a 95% confidence interval, 5% absolute precision and 40.1% (Birhanu *et al.*, 2017) expected prevalence, as follows (Thrusfield, 2005):

$$n = \frac{1.96^2 P_{exp}(1-P_{exp})}{d^2}$$

Where, P_{exp} was expected prevalence, d absolute precision and n sample size. Accordingly, the calculated sample size was 221 animals. However 316 lactating cows were included in the study to increase precision and make the data representative.

Data collection

Data on the risk factors like age, parity, stage of lactation, udder hygiene, farm hygiene, presence of injury on the skin of udder/teat, and previous mastitis history were collected in a properly designed format.

Clinical examination

The udder was examined through visual inspection and palpation to detect possible abnormalities like swelling or decrease in size, pain, swelling of supra mammary lymph nodes, disproportional symmetry of teats, and blindness. Likewise, the milk was examined for discoloration and the presence of clots, flakes, blood, and watery secretions to detect clinical mastitis.

California mastitis test

The California mastitis test was carried out according to the method described by Quinn *et al*. (1999). A squirt of milk, about 2 ml from each quarter was placed in each of four shallow cups in the CMT paddle. An equal amount of the commercial reagent (Immuncell 56 Evergreen Drive, Portland, ME 04103) was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane for 15 seconds. The result was scored from 0-3 and Score 0 (N) was considered negative and T (Trace), 1, 2 and 3 were considered positive for subclinical mastitis according to Quinn *et al*. (1999).

Milk sample collection

Milk samples from both forms of mastitis were collected according to the National Mastitis Council (NMC) (1990). The teats were cleaned with 70% ethyl alcohol, and approximately 10 ml of milk were collected in to a sterile test tube after discarding the first 3 milking streams. Then, samples were placed in racks for ease of handling and transported in an ice to Addis Ababa University, College of Veterinary Medicine and Agriculture, Microbiology Laboratory, and on arrival, it was inoculated on a standard bacteriological media for isolation and characterization of bacteria (Biru, 1989; NMC, 1990) and for milk composition analysis the milk was taken to Ethiopian Meat and Dairy Industry Development Institute (EMDIDI).

Bacteriological isolation

Milk samples were bacteriologically examined according to the procedures employed by Quinn *et al.* (1999). A loopful of milk samples collected from each infected quarter was inoculated separately on MacConkey agar and blood agar enriched with 5% defibrinated sheep blood. The inoculated plates were then incubated aerobically at 37 °C for 24 to 48 hours.

The identification of the bacteria on the primary culture was made on the basis of colony morphology, hemolytic characteristics, and Gram stain reaction, including the shape and arrangements of the bacteria, as well as catalase and O-F tests. Staphylococci were identified based on catalase test, growth characteristics on mannitol salt agar and purple agar, and tube coagulase test. Identification of streptococci was made according to growth characteristics on Edward's media, catalase test, hydrolysis of Esculin, and CAMP test. Gram negative isolates grown on MacConkey agar were identified based on growth characteristics on MacConkey agar, oxidase reaction, catalase test and the "IMViC" (Indole, Methyl red, Voges-Proskaur and Citrate) test (Quinn *et al*., 1999).

Chemical analysis of milk

Milk samples collected from both mastitis positive and negative cows (controls) were brought to Ethiopian Dairy and Meat Industry Development Institute (EDMIDI) milk chemistry laboratory and analyzed using Lactoscan Ultrasonic milk analyzer machine according to the manufacturer's (Milkotronic Ltd, Nova Zagora, Bulgaria) instructions. The pH was measured by a digital pH-meter, as described by Aggad *et al.* (2010).

Data analysis

Data were entered into Microsoft Excel sheet and analyzed using STATA software version 13. The Chi-square test was used to assess the association of factors with mastitis; two samples mean comparison *t*-test was used to compare means in milk composition of mastitis positive and negative cows. A difference was taken as significant at *p*-value less than 0.05 and the confidence level was held at 95%.

Results

Prevalence of mastitis

Out of 316 cows examined, 230 (72.8%) were positive for bovine mastitis, of which 30 (9.5%) were positive for clinical and 200 (63.3%) were positive for subclinical mastitis, as presented in Table 1. Out of 1264 quarters, 36 (2.85%) were blind teats and non-functional. Out of 1228 quarters with functional teats, 555 (45.2%) were mastitis positive, as presented in Table 2.

Table 1. Prevalence of clinical and subclinical mastitis.

Forms of mastitis	Number of cows examined	Number positive	Prevalence (%)
Clinical	316	30	9.5
Subclinical	316	200	63.3
Total	316	230	72.8

Table 2. Prevalence of mastitis at quarter level.

Quarter	Number of quarters examined	Number positive	Prevalence (%)	Blind teats	Prevalence (%)
Left front	304	139	45.72	12	3.79
Right front	306	140	45.75	10	3.16
Left rear	310	147	47.42	6	1.89
Right rear	308	129	41.88	8	2.53
Total	1228	555	45.2	36	2.85

Association of risk factors with prevalence

The potential risk factors considered for the occurrence of bovine mastitis were age, herd size, number of parity, stage of lactation, udder hygiene, farm hygiene, presence of injury on the skin of the udder/teat, and previous mastitis history. A statistically significant association ($p<0.05$) was observed in cows with poor udder and farm hygiene and cows with teat injury, as presented in Table 3.

Risk factors like udder hygiene, presence of teat/udder injury, and previous mastitis showed significant association ($p<0.05$) with the prevalence of clinical

mastitis. Udder and farm hygiene showed significant association (p<0.05) with the prevalence of subclinical mastitis, as presented in Table 4.

Table 3. Association of overall prevalence of bovine mastitis with some potential risk factors.

Risk factors	Nº of cows examined	Nº positive (%)	X^2	*p* value
Age				
Young adult (3-6 years)	206	149 (47.15)	0.0618	0.804
Adult (≥7 years)	110	81 (25.63)		
Herd Size				
Few (<10)	152	106 (33.54)	1.3736	0.241
Many (≥10)	164	124 (39.24)		
No of Parity				
Few (1-3)	148	104 (32.91)	0.8886	0.346
Many (≥4)	168	126 (39.87)		
Stage of lactation				
Early (1-3 months)	120	89 (28.16)		
Mid (4-6 months)	90	65(20.57)	0.1933	0.908
Late (≥7 months)	106	76(24.05)		
Udder hygiene				
Good	131	72(22.78)	35.8835	0.000
Poor	185	158(50)		
Farm hygiene				
Good	115	51(16.14)	73.8081	0.000
Poor	201	179(56.65)		
Teat/udder injury				
Absent	288	205(64.87)	4.2230	0.040
Present	28	25(7.91)		
	190	136(43.04)	0.3498	0.554
	126	94(29.75)		

X2 : Chi-square

Table 4. Association of clinical and subclinical mastitis with some risk factors.

Risk factors	Nº of cows examined	Clinical mastitis			Subclinical mastitis		
		No positive (%)	X2	*p* value	No positive (%)	X2	*p* value
Age							
Young adult (3-6 years)	206	16 (5.06)	2.053	0.152	133 (42.09)	0.4121	0.521
Adults (≥7 years)	110	14 (4.43)			67 (21.20)		
Herd size							
Few (<10)	152	10 (3.16)	2.895	0.089	96 (30.38)	0.0022	0.962
Many (≥10)	164	20 (6.33)			104 (32.91)		
No of parity							
Few (1-3)	148	14 (4.43)	0.000	0.984	90 (28.48)	0.7371	0.391
Many (≥4)	168	16 (5.06)			110 (34.81)		
Stage of lactation							
Early (1-3 months)	120	6 (1.90)	4.795	0.091	83 (26.27)	3.0186	0.221
Mid (4-6 months)	90	10 (3.16)			55 (17.41)		
Late (≥7 months)	106	14 (4.43)			62 (19.62)		
Udder hygiene							
Good	131	4 (1.27)	10.801	0.001	68 (21.52)	12.478	0.000
Poor	185	26 (8.23)			132 (41.77)		
Farm hygiene							
Good	115	6 (1.90)	3.847	0.050	45 (14.24)	45.424	0.000
Poor	201	24 (7.59)			155 (49.05)		
Teat/udder injury							
Absent	288	20 (6.33)	24.582	0.000	185(58.54)	1.2492	0.264
Present	28	10 (3.16)			15(4.75)		
Previous mastitis							
No	190	10 (3.16)	9.925	0.002	126 (39.87)	1.8763	0.171
Yes	126	20 (6.33)			74 (23.42)		

X2: Chi-square

Bacterial isolates

A total of 80 milk samples from distinct and strong positive quarters for CMT were cultured. From 80 milk samples cultured, 58 (72.5%) yielded single bacterial colony, 7 (8.75%) mixed colonies and 15 samples had no growth. In this study, *Staphylococcus aureus* was the most frequently isolated species

(11.25%), followed by *Escherichia coli* (10%), *Micrococcus* species (10%) and *Pseudomonas aeruginosa* (10%), as presented in Table 5.

Table 5. Bacterial species isolated from bovine mastitis in the study area.

Bacteria	Frequency	Prevalence (%)
Staphylococcus aureus	9	11.25
Escherichia coli	8	10
Micrococcus species	8	10
Pseudomonas aeruginosa	6	7.5
Coagulase negative *Staphylococcus* (CNS)	5	6.25
Streptococcus agalactiae	5	6.25
Enterobacter aerogenes	5	6.25
Klebsiella pneumonae	4	5
Streptococcus disgalactiae	2	2.5
Streptococcus uberis	2	2.5
Staphylococcus intermedius	2	2.5
Bacillus species	2	2.5
Mixed growth	7	8.75
No growth	15	18.75
Total	80	100

Chemical analysis of milk composition

A total of 80 milk samples (50 from distinct and strong CMT positive and 30 from CMT negative cows) were analyzed using Lactoscan Ultrasonic milk analyzer machine for fat, protein, solid non-fat (SNF), density, and freezing point of the milk. The pH meter was used to measure pH. The difference in pH, protein, SNF, and freezing point showed statistically significant difference ($p< 0.05$) between mastitis-positive and negative milk. Mastitis-positive milk had higher pH value than negative milk. Protein and SNF content of mastitis positive milk were lower than negative milk, as presented in Table 6.

Table 6. Variations in milk composition between mastitis positive and negative samples.

Analytes	Mastitis	Mean ± SE	95% CI	*p* value
pH	Positive	6.316 ± 0.061	6.194 - 6.439	0.000
	Negative	5.897 ± 0.041	5.813 - 5.980	
Fat	Positive	4.330 ± 0.281	3.914 - 4.747	0.092
	Negative	3.867 ± 0.207	3.293 - 4.441	
Protein	Positive	2.966 ± 0.042	2.844 - 3.087	0.000
	Negative	3.278 ± 0.060	3.190 - 3.363	
SNF*[1]	Positive	6.924 ± 0.168	6.587 - 7.261	0.000
	Negative	7.836 ± 0.118	7.595 - 8.077	
Freezing point	Positive	-0.493 ± 0.009	-0.513 - 0.473	0.000
	Negative	-0.550 ± 0.007	-0.564 - 0.536	
Density (g/ml)	Positive	1.023 ± 0.002	1.029 - 1.030	0.162
	Negative	1.029 ± 0.001	1.028 - 1.030	

SNF: Solid not fat; SE: Standard error; CI: Confidence interval

Discussion

In the present study the overall prevalence of mastitis was 72.8%, which was comparable with previous studies of Mekbib *et al.* (2010) (71.0%) in Holeta and Zeryehun *et al.* (2013) (74.7%) in Addis Ababa, Ethiopia. However, it was higher than the findings of Sori *et al.* (2005) and Abunna *et al.* (2013) who reported 57.7% in Sebeta and 52.27% in Addis Ababa, respectively.

The prevalence of clinical mastitis in the current study (9.5%) was comparable with previous findings of Delelesse (2010) (10.3%) and Mekbib *et al.* (2010) (10%) in Holeta, Ethiopia, but lower than the reports of Workineh *et al.* (2002) (25.1%) and Zeryehun *et al.* (2013) (19.6%) in Addis Ababa. The prevalence of subclinical mastitis in this study was comparable with Fesseha (2021), who reported 71.02% in Modjo, Ethiopia, but higher than the findings of Adugna (2008), who reported 18.9% in Dire Dawa and Haramaya University Dairy farm and Gizat (2004), who reported 17% in and around Bahir Dar. The variations in prevalence may exist as a result of differences in the management of farms, especially with respect to the prevailing sanitary conditions at the time of investigation, agro-climatic variation, selection criteria and number of animals. Mastitis is a complex disease involving interactions of various factors

such as husbandry practices and environmental conditions (Radostits *et al.,* 2007).

The quarter level prevalence in the current study (45.2%) was comparable with Mekbib *et al.* (2010), who reported 44.9% in Holeta. However, it was lower than findings by Kifle and Tadele (2008), who reported quarter prevalence rate of 63.1%. There was no significant difference (p>0.05) among the quarters in occurrence of bovine mastitis.

In this study, subclinical mastitis has been found to be higher than clinical mastitis. This could be attributed to the little attention given to subclinical mastitis while treating clinical cases. Moreover, farmers may need to be better informed about the impact of the silent cases of mastitis (Karimuribo *et al.*, 2006).

The presence of injury on the skin of the udder/teat, udder, and farm hygiene showed statistically significant (p< 0.05) association with the occurrence of bovine mastitis. These findings show a strong agreement with the findings of Biffa *et al.* (2005), who reported association of bovine mastitis with presence of injury and Zeryehun *et al.* (2013), who reported association of bovine mastitis with udder hygiene. This shows that poor udder and farm hygienic condition and udder injuries may lead to increased mastitis prevalence and, consequently, result in reduced milk quality and quantity .

The most frequent bacterial species isolated from mastitis-positive milk was *S. aureus*. Radostits *et al.* (2007) asserted that *S. aureus* is well adapted to survive in the udder. It usually establishes a mild sub clinical infection of long duration from which it is shed in milk, facilitating transmission to healthy animals mainly during milking. In the present study, the frequency of isolation of *E. coli* was quite high, and it could be due to poor hygiene condition, as it infects udder through teat canal (Sumathi *et al*., 2008). 18.75% of milk samples from CMT- positive cows did not yield any bacterial growth. This might be due to the elimination of the infection although the SCC has still not declined (Sandholm, 1995). Anaerobes and, fungi may also cause mastitis, and it is probably more rarely caused by non-infectious causes (Hogan *et al*., 1999). The predominant isolation of *Staphylococcus aureus* (11.25%) in the present study showed an agreement with previous reports of Mekbib *et al.* (2010), Zeryehun *et al.* (2013), Atyab *et al.* (2006), and Fadlelmoula *et al.* (2007), who reported higher prevalence of *Staphylococcus aureus*.

The pH, total protein, solid not fat, and freezing point showed significant difference ($p < 0.05$) between CMT-positive and negative samples. The pH was significantly ($p < 0.05$) higher in CMT-positive milk; the result was in agreement with Riaz *et al.* (2012), Batavani *et al.* (2007), Charjan *et al.* (2000) and Haggag *et al.* (1991) and in disagreement with Ogola *et al.* (2007) who reported non-significant increase in pH of positive milk. These change were due to the increase in permeability of the mammary epithelial cells that lead to the transfer of components from blood to milk, such as citrates, bicarbonates that cause elevated pH levels (Harmon, 1994) and due to reduction in lactose contents as the lactic acid formation is minimum (Tanveer *et al.*, 2005). The milk pH of non mastitic cows in this study decreased from normal pH range, and this might have resulted from formation of lactic acid due to unwanted delay to analyze milk samples immediately after collection.

Total milk protein content was significantly ($p<0.05$) lower in milk from positive animals than negative animals; this result was in agreement with that of Jones (2006) and but was in disagreement with the results of Sonea *et al.* (2009) and Auldist and Hubble (1998), who reported significant elevation of milk protein due to mastitis. This study showed a decrease in the protein content of milk from mastitic cows. This might be due to high increase in the activity of proteolytic enzyme (plasmin) that causes extensive destruction of milk proteins in the udder before milk removal (Khan and Khan, 2006).

Solid not fat (SNF) was significantly ($p<0.05$) lower in mastitis-positive milk compared with normal milk. Decrease in SNF in positive milk depends on the destruction that occurs due to the invasion of pathogens to the mammary tissue causing a decrease in synthetic activity of mammary gland (Benchedly *et al.*, 2009). The result was in accordance with findings of Riaz *et al.* (2012), Ahmed *et al.* (2007) and Malek *et al.* (2013) but disagreed with Leitner *et al.* (2004) and Sefinew *et al.* (2013), who reported that mastitis had no significant effect on the solid not fat content of milk.

In this study, the freezing point of mastitic milk significantly increased when compared with normal milk. This might be attributed to significant decrease in protein and SNF content of the milk. The chemical composition of milk had influence on freezing point, as Navrátilová *et al.* (2006) found correlation between freezing point and chemical composition of milk.

Conclusion

Bovine mastitis is the major constraint to the agricultural sector in general and dairy production in particular. The present study revealed that bovine mastitis was highly prevalent disease in the study area. Cows kept in farms with poor hygiene and hence contaminated udder and teat/udder injuries were predisposed to mastitis than those kept in relatively clean farms. As the study revealed, subclinical mastitis was more prevalent than clinical mastitis. Also, milk composition was affected in cows with mastitis, and a variety of aerobic bacterial pathogens were isolated from milk of mastitic cows. These pathogens could be the cause of mastitis. Most of these pathogens were contagious and hence, might easily contaminate other cows in the herd. Dairy production and health extension programs should give special attention to controling bovine mastitis as it is a serious challenge for the dairy industry in the study area. Improved sanitary measures with particular emphasis to udder/teat and farm hygiene should be implemented in all dairy farms. Regular screening and early treatment of mastitic cows can reduce dispersion of pathogens within the herd. In this regards, antibiotic sensitivity tests are recommended before treatment to obtain maximum efficacy. Farms should avoid as much as possible and pay attention to wounds on the udder/teats and get immediate veterinary assistance before it leads to clinical mastitis.

References

Abebe, R., Abera, M., Denbarga, Y., Suleyman, M., Fekadu, A., Abunna, F., *et al*., 2020. Prevalence, risk factors and bacterial causes of bovine mastitis in southern Ethiopia. *Ethiop. Vet. J.*, 24(1), 52–68. doi:10.4314/evj.v24i1.4.

Abunna, F., Megersa, B. and Regassa, A., 2013. Bovine mastitis: prevalence, risk factors and bacterial isolation in small-holder dairy farms in Addis Ababa city, Ethiopia. *Glob. Vet*., 10, 647-52.

Adugna, B., 2008. Cross sectional study of mastitis in Dire Dawa and Haramaya University dairy farms, prevalence isolation and identification of pathogens and Antimicrobial sensitivity testing, Eastern Ethiopia. DVM Thesis, FVM, Haramaya University, Ethiopia.

Aggad, H., Bridja, M., Bouhai, A., Benaouali, M. and Djebli, A., 2010. Some quality aspects of pasteurized milk in Algeria. *World J. Dairy Food Sci*, 5(1), 21-24.

Ahmed, T., Bilal, M., Uallah, S., Rahman, Z.U. and Muhammad, G., 2007. Impact of mastitis severity on mineral contents of buffalo milk. *Pak. J. Agric. Sci*., 44, 176-78.

Albenzio, M., Taibi, L., Muscio, A. and Sevi, A., 2002. Prevalence and etiology of subclinical mastitis in intensively managed flocks and related changes in the yield and quality of milk. *Small Rumin. Res.*, 43, 219-26.

Al-Majali, A.M., Al-Qudah, K.M., Al-Tarazi, Y.H. and Al-Rawashdeh, O.F., 2008. Risk factors associated with camel brucellosis in Jordan. *Trop. Anim. Health Prod.*, 40, 193-200.

Amin, B., Deneke, Y. and Abdela, N., 2017. Bovine mastitis: prevalence, risk factors and isolation of *Streptococcus* species from small holders dairy farms in and around Haramaya town, eastern Ethiopia. *Global J. Med. Res.*, 17(1), 27–38.

Anon, R., 2001. Veterinary Investigation Surveillance Report. London, Veterinary Laboratories Agency.

Atyab, N., Vodjgani, M., Gharagozloo, F. and Bahonar, A., 2006. Prevalence of bacterial mastitis in cattle from the farms around Tehran. *Iranian J. Vet. Res.*, 7(3), 76-79.

Auldist, M.J. and Hubble, I.B., 1998. Effects of mastitis on raw milk and dairy products. *Aust. J. Dairy Tech.*, 53(1), 28-36.

Batavani, R.A., Asri, S. and Naebzadeh, H., 2007. The effect of subclinical mastitis on milk composition in dairy cows. *Iran J. Vet. Res.*, 8, 205-11.

Belachew, T., 2016. Bovine mastitis: prevalence, isolation of bacterial species involved and its antimicrobial susceptibility test around Debrezeit, Ethiopia. *J. Vet. Sci. Technol.*, 7, 396.

BenChedly, H., Boutinaud, M., Bernier-Dodier, P., Marnet, P. G. and Lacasse, P., 2009. Disruption of cell junctions induces apoptosis and reduces synthetic activity in lactating goat mammary gland. *J. Dairy Sci.*, 93, 2938-51.

Biffa, D., Debela, E. and Beyene, F., 2005. Prevalence and risk factors of mastitis in lactating dry cows in southern Ethiopia. *Int. J. Appl. Res. Vet. Med.*, 3(3), 189-98.

Birhanu, M., Leta, S., Mamo, G. and Tesfaye, S., 2017. Prevalence of bovine subclinical mastitis and isolation of its major causes in Bishoftu town, Ethiopia. *BMC Res. Notes*, 10 (1), 767.

Biru, G., 1989. Major bacteria causing bovine mastitis and their sensitivity to common antibiotics. *Ethiop. J. Agri. Sci.*, 11, 4-6.

Bradley, A. J., 2002. Bovine mastitis: an evolving disease. *Vet. J.*, 163, 1-13.

Charjan-Ku, P.Y., Mangle, N.S., Kalorey, D.R. and Kuralkar, S.V., 2000. Changes in milk pH and the levels of Na and K in whey associated with udder health status of cow. *Indian Vet. J.*, 77(12), 1066-68.

CSA, 2012. The 2012 population projection from 2007 population census. Central Statistical Agency of the Federal Democratic Republic of Ethiopia, Addis Ababa, Ethiopia.

CSA, 2019. Agricultural Sample Survey. Vol. II Report on Livestock and Livestock characteristics. Statistical Bulletin 588. Addis Ababa, Central Statistical Agency of the Federal Democratic Republic of Ethiopia.

Delelesse, G.D., 2010. Study on prevalence of bovine mastitis on cross breed dairy cow around Holeta areas, West Shoa Zone of Oromia, Ethiopia. *Global Vet*., 5(6), 318-23.

Eriskine, R.J., 2001. Intramuscular administration of ceftiofur sodium versus intrammamary infusion of penicillin/novobiocin for treatment of *Streptococcus agalactiae* mastitis in dairy cows. *J. Am. Vet. Med. Assoc*., 208(2), 258-60.

Fadlelmoula, A., Fahr, R.D., Anacker, G. and Swalve, H.H., 2007. The management practices associated with prevalence and risk factors of mastitis in large scale dairy farms in Thuringia-Germany 1: environmental factors associated with prevalence of mastitis. *Aust. J. Basic Appl. Sci.*, 1(4), 619-24.

Fekadu, K., 1995. Survey on the prevalence of bovine mastitis and the predominant causative agents in Chaffa valley. In: Proceedings of the 9th Conference of Ethiopian Veterinary Association: Addis Ababa, Ethiopia, pp. 101-11.

Fesseha, H., Mathewos, M., Aliye, S. and Wolde, A., 2021. Study on prevalence of bovine mastitis and associated risk factors in dairy farms of Modjo town and suburbs, central Oromia, Ethiopia. *Vet. Med.: Res. Rep*., 12, 271-83.

Gianneechini, R., Concha, C., Rivero, R., Delucci, I. and López, M.J., 2002. Occurrence of clinical and sub-clinical mastitis in dairy herds in the West Littoral Region in Uruguay. *Acta. Vet. Scand*. 43, 221.

Gizat, A., 2004. A cross sectional study of bovine mastitis in and around Bahir Dar and antibiotics resistance patterns for major pathogens. MSc Thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debrezeit, Ethiopia.

Haggag, H.F., Hamzawi, L.F., Mahran, G.A. and Ali, M.M., 1991. Physico-chemical properties of colostrum and clinical and sub-clinical mastitic buffalo milk. *Egypt. J. Dairy Sci*., 19(1), 55-63.

Harmon, R.J., 1994. Physiology of mastitis and factors affecting somatic cell count. *J. Dairy Sci*., 77, 2103-12.

Hogan, J.S., Gonzalez, R.N., Harmon, R.J., Nickerson, S.C., Oliver, S.P., Pankey, J.W., *et al*., 1999. Laboratory handbook on bovine mastitis. National Mastitis Council, Madison, WI, 78(7), 485-88.

Jones, G.M., 2006. Understanding the basics of mastitis. Virginia cooperative Extension. Publication No. 404-233. Virginia State University, USA, pp. 1-7.

Karimuribo, E.D., Fitzpatrick, J.L., Bell, C.E., Swai, E.S., Kambarage, D.M., Ogden, N.H., *et al*., 2006. Clinical and subclinical mastitis in smallholder dairy farms in Tanzania: Risk, intervention and knowledge transfer. *Prev. Vet. Med*., 74, 84-98.

Khan, M. Z. and Khan, A., 2006. Basic facts of mastitis in dairy animals. *Pak. Vet. J.*, 26(4), 204-208.

Kifle, A. and Tadele, T., 2008. Prevalence of sub clinical mastitis in small holder dairy farms in Selale, North Shewa Zone, Central Ethiopia. *Internet J. Vet. Med*., 5(1), 1-4.

Korhonen, H. and Kaartinen, L., 1995. Changes in the composition of milk induced by mastits. In: The bovine udder and mastitis. Sandholm, M., Honkanen-Buzalski, T., Kaartinen, L. and Pyörälä, S. (eds.), University of Helsinki, Finland. Pp. 76-82.

Leitner, G., Merin, U. and Silanikove, N., 2004. Changes in milk composition as affected by sub clinical mastitis in goats. *J. Dairy Sci*., 87, 1719-26.

Malek, C.B, Barreiro, J.R, Mestieri, L., Porcionato, M.A. and Santos, M.V., 2013. Effect of somatic cell count and mastitis pathogens on milk composition in Gyr cows. *BMC Vet. Res*., 9, 67. doi: 10.1186/1746-6148-9-67.

Mekbib, B., Furgasa, M., Abunna, F., Megersa, B. and Regassa, A., 2010. Bovine mastitis: prevalence, risk factors and major pathogens in dairy farms of Holeta town, central Ethiopia. *Vet. World*, 3, 397-403.

Mohamed, A., Simeon, E. and Yemesrach, A., 2004. Dairy development in Ethiopia. International Food Policy Research Institute, EPTD Discussion Paper No. 123. Washington, DC, U.S.A.

Navrátilová, P., Janštová, B., Gloss O.P. and Vorlová, L., 2006. Freezing point of heat-treated drinking milk in the Czech Republic. *Czech J. Food Sci*., 24(4), 156-63.

NMC, 1990. Microbiological procedures for the diagnosis of bovine udder infection, 3rd ed. National Mastitis Council, Arlington, pp. 1-15.

Ogola, H., Shitandi, A. and Nanua, J., 2007. Effect of mastitis on raw milk compositional quality. *J. Vet. Sci*., 8, 237-42.

Quinn, P.J., Carter, M.E., Markey, B. and Carter, G.R., 1999. Clinical Veterinary Microbiology. Mosby Publishing, London. pp. 327-44.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D., 2007. Mastits. In: Veterinary Medicine: A text book of disease of cattle, sheep, pigs, goats, and horses, 10th ed., Ballier, Tindall, London. pp. 674-762.

Rerk-u-suke, S., Samngamnim, S., Koowatananukul, C. and Ajariyakhajorn, K., 2008. Effect of Streptococcus uberis Causing Intramammary Infection on Raw Milk Compositions. In: Proceedings, the 15th Congress of FAVA, 27-30 October, Bangkok, Thailand FAVA – OIE Joint Symposium on Emerging Diseases.

Riaz, H., Muhammad, T.J. and Ahrar, K., 2012. Changes in Some Biochemical Parameters and Somatic Cell Counts in the Milk of Buffalo and Cattle Suffering from Mastitis. *Pak. Vet. J*., 32(3), 418-21.

Sandholm, M., 1995. Inflammation in Mastitis. In: Sandholm, M., Honkanen-Buzalski, T., Kaartinen, L. & Pyörälä. S. (ed.). University of Helsinki, Faculty of Veterinary Medicine, The Bovine Udder and Mastitis, pp. 59-75. Gummerus Kirjapaino, Jyväskylä (ISBN 951-834-047-1).

Santos, J.E., Cerri, R.L., Ballou, M.A., Higginbotham, G.E. and Kirk, J.H., 2004. Effect of timing of first clinical mastitis occurrence on lactational and reproductive performance of Holstein dairy cows. *Anim. Reprod. Sci*., 80, 31-45.

Sefinew, A., Firaol, T., Gizat A. and Awoke, T., 2013. Study on bovine mastitis and its effect on chemical composition of milk in and around Gondar Town, Ethiopia. *J. Vet. Med. Anim. Health*, 5(8), 215-21.

Sonea, C., Colceri, D. and Bacila, V., 2009. Research on sub-clinical mastitis effect on milk quality. *Zooteh. Biotehnol*., 42(2).

Sori, T., Zerihun, A. and Abdicho, S., 2005. Dairy cattle mastitis in and around Sebeta, Ethiopia. *Int. J. Appl. Res. Vet. Med.*, 3(4), 332-38.

Sumathi, B.R., Veeregowda, B.M. and Amitha, R.G., 2008. Prevalence and antibiogram profile of bacterial isolates from clinical bovine mastitis. *Vet. World*, 1, 237.

Tanveer, A., Muhammad, Q. B., Shafiq, U. and Muhammad, G., 2005. Effect of severity of mastitis on pH and specific gravity of buffalo milk. *Pak. J. Agri. Sci*., 42, 3-4.

Tesfaye, B. and Abera, A., 2018. Prevalence of mastitis and associated risk factors in Jimma town dairy farms, western Ethiopia. *J. Vet. Sci. Anim. Husb*., 6(3), 307.

Thrusfield, M., 2005. Veterinary Epidemiololgy, 3rd Edition, Blackwell Publishing Professional, Ames, Iowa, USA. W.B. Saunders, London, pp. 451-452.

Wakeman, D. and Pace, J., 1983. Determining the age of cattle by their teeth. *J. Am. Vet. Med. Assoc*. 121, 483.

Workineh, S., Bayleyegne, M., Mekonnen, H. and Potgieter, L.N.D., 2002. Prevalence and aetiology of mastitis in cow from two major Ethiopian dairies. *Trop. Anim. Health Prod*., 34, 19-25.

Zeryehun, T., Aya, T. and Bayecha, R., 2013. Study on prevalence, bacterial pathogens and associated risk factors of bovine mastitis in small holder dairy farms in and around Addis Ababa, Ethiopia. *J. Anim. Plant Sci*., 23, 50-55.