Prevalence, risk factors and bacterial causes of bovine mastitis in southern Ethiopia

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Abstract

Mastitis is one of the most economically important diseases in dairy farms worldwide. It is particularly important in Ethiopia where no routine prevention and control practices are in place. This cross-sectional study was carried out between October 2017 and June 2018 to estimate the prevalence of mastitis, identify the associated risk factors and isolate bacterial causes in dairy farms located in southern Ethiopia using standard microbiological methods and questionnaire survey. A total of 686 lactating cows which were found in 122 selected dairy farms were investigated by physical examination and California mastitis test. The overall cow-level prevalence of mastitis was 54.2% (95% CI: 50.5 – 57.9%). Based on the study site, the prevalence was 55.7% in Hawassa, 54.3% in Arsi Negele, 52.6% each in Wondo Genet and Wolayta Soddo towns with no significant ($p > 0.05$) difference among the sites. The majority of mastitis cases were subclinical (48.1%) while the clinical mastitis was only 6.1%. Of the 122 herds tested, 109 (89.3%; 95% CI: 82.1 – 93.9%) had at least a cow positive for mastitis. The study showed that high parity number (OR = 1.6; $p = 0.015$), flat (OR = 4.5; $p < 0.001$) and round (OR = 2; $p < 0.001$) teat end shape, history of mastitis in preceding lactation (OR = 3.3; $p < 0.001$), and slightly (OR = 3.5; $p < 0.001$), moderately (OR = 4.9; $p < 0.001$), and very dirty (OR = 9.2; $p < 0.001$) udder and legs were the major risk factors which are significantly associated with higher prevalence of mastitis. Based on the available media and reagents, the major bacteria isolated from subclinical mastitic milk samples were *Staphylococcus* spp. (57.3%), *Streptococcus* spp. (18.6%), *E. coli* (17.3%) and *Bacillus* spp. (7.5%) in order of their abundance. The present study revealed a high prevalence of mastitis, particularly the subclinical one, and the associated risk factors. Enhancing the awareness of dairy farmers, regular screening of cows for subclinical mastitis, proper treatment of the clinical cases, improving the hygienic condition of the cows, and culling of chronically infected cows are critically important to prevent and control bovine mastitis.

Keywords: Mastitis, Prevalence, Risk factors, Southern Ethiopia

Introduction

Mastitis is an inflammation of the parenchyma of mammary glands characterized by physical, chemical and usually, bacteriological changes in milk and pathological changes in glandular tissues. The most important changes in the milk include discoloration, the presence of milk clots and large number of leucocytes in milk (Radostits *et al*., 2007). It is a multi-etiological and complex disease resulting from the interaction of three major factors: infectious agents, host resistance, and environmental factors (Gera and Guha, 2011).

Mastitis is a global problem adversely affecting animal health, quality of milk and the economics of milk production (Sharma and Sindhu, 2007). It is the most widespread infectious disease in dairy cattle (Tiwari et al., 2000; Elango et al., 2010; Sharma et al., 2012). Mastitis can occur either in clinical or subclinical forms. The clinical mastitis is characterized by changes in the udder and milk that are directly observable, whereas the subclinical mastitis is characterized by an increase in somatic cell count in the milk and absence of visible clinical signs (Kivaria et al., 2004).

Mastitis is of a particular concern for farmers in developing countries like Ethiopia. In Ethiopia, there are several bovine mastitis studies showing spatial variations in prevalence and risk factors. According to most recent studies, the prevalence of mastitis ranges from 39.5 – 62.6% (Belayneh *et al*., 2013; Tolosa *et al*., 2013; Abebe *et al.,* 2016; Birhanu *et al*., 2017). Since Ethiopia is a country with diverse agro-ecological conditions, it is obvious that mastitis prevalence and associated risk factors can vary from region to region. Thus, it is important to investigate the causes and risk factors of the disease in parts of southern Ethiopia to formulate mastitis control program adapted to the specific local situation. The present study aims to estimate the prevalence of bovine mastitis, associated risk factors, and identify the major bacterial causes of mastitis in dairy farms in southern Ethiopia.

Materials and Methods

Study area

The study was conducted in small scale dairy farms located in Hawassa, Wendo Genet, Wolayita Sodo and Arsi Negelle towns which are high potential areas for dairy production in southern Ethiopia. The first three towns are found in Southern Nations, Nationalities and People's Regional State (SNNPRS) whereas Arsi Negelle is in Oromiya Regional State. Hawassa is located 275Kms south of Addis Ababa at 7º3´N latitude and 38º48´E longitude. It is situated at an elevation of 1708 meters above sea level. Hawassa receives an average annual rain fall of 900 mm and has mean annual temperature of 20°C. Wendo Genet is located at 30 Kms west of Hawassa. It is situated at about 1723 meters above sea level, 7°5'N latitude and $38^0$37'E longitude. The average annual rainfall of the town is 1372 mm while mean annual temperature is 19 °C. Wolayta Sodo is situated at 6°54′N latitude and 37°45′E longitude, and has an elevation between 1600 and 2100 meters above sea level. The average annual rainfall of the town ranges from 450 mm to 1446 mm while the mean annual maximum and minimum temperature of the town are 26.6 ^{0}C and 11.4 ^{0}C, respectively. Arsi Negelle is found in the West Arsi zone of the Oromia regional state at a distance of 225 Kms from Addis Ababa. The town is situated at about 2043 meters above sea level at 7°21'N latitude and 38°42'E longitude. The average annual temperature of the area varies from 10 to 25 °C while rainfall varies between 500 and 1000 mm.

Study population

The study population covers lactating dairy cows raised under semi-intensive or intensive management system. In intensive farms, cattle were kept indoors all the time and provided with roughages and concentrates. The semi-intensive farms are characterized by outdoor grazing at day time and provision of supplementary feed in the morning and evening before milking. House construction design in the study areas also varied from farm to farm. In some of the farms, the wall was made of bricks while in others it was built from wood and mud. The floors of houses were constructed from concrete, wood or soil compact with or without beddings. In most of the houses, drainage system was not sufficient enough to remove slurry. Although it was difficult to get the actual figure, the approximate number of dairy farms found in the towns were 107 in Hawassa (Libiyos, 2018, unpublished data), 35 in Arsi Negelle (Teherku, 2018, unpublished data), 33 in Wolayta Sodo (Dema, 2018, unpublished data) and 63 in Wendo Genet (Wendo Genet Wereda Livestock and Fisheries Development Office, 2018, unpublished data). The size of the herds in the study towns ranged from 2 to 131 cattle with average herd size of 7 cattle per herd.

Study design and sample size

The study employed a cross-sectional study design. The required sample size was determined using the recommended formula (Thrusfield, 2005) with 95% confidence interval, 5% absolute precision, 0.15 between cluster variance (Vc) and 62.6% expected cow level prevalence (Abebe *et al.*, 2016). Based on the given formula, the number of farms calculated was 122 and with predicted average number of five cows per farm, the minimum sample size was determined as 610 cows. The sample size was allocated proportionally to each of the study areas based on their dairy cattle population size. Accordingly, a sample size of 305, 152, 135, and 94 dairy cows was allocated for Hawassa, Wolayta Sodo, Wendo Genet, and Arsi Negelle towns, respectively. The dairy farms were selected randomly but all lactating cows found in the selected farms were included in the study.

Clinical examination

A thorough physical examination of the udder and teats was conducted on all lactating cows for evidence of clinical mastitis. Clinical findings such as secretions, abnormalities on size and shape of the udder, its consistency and temperature were assessed by visual inspection and palpation. Then, the cows negative for clinical mastitis were subjected to California Mastitis Test (CMT) for detection of subclinical mastitis. CMT was carried out according to the procedure described by Quinn et al (2002). In brief, a squirt of milk, about 2 ml from each quarter, was placed in each of the four shallow cups in the CMT paddle. An equal amount of CMT reagent was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane for 15 seconds. The CMT result was scored as negative (0 and trace), 1 (weak positive), 2 (distinct positive) and 3 (strong positive) based on gel formation.

Bacteriological analysis

A total of 307 milk samples were collected from sub clinically affected (CMT positive) cows aseptically based on the procedure described in NMC (1999). The teats were wiped thoroughly with 70% ethyl alcohol and approximately 10 ml of milk were collected into a sterile bottle after discarding the first 3 milking stream. After collection, samples were transported in an icebox to Microbiology Laboratory, Faculty of Veterinary Medicine of Hawassa University. In the laboratory, samples were cultured immediately or stored at +4°C for a maximum of 24 hours until inoculated on a standard bacteriological media (NMC, 1999).

A loopful of the milk samples was streaked on blood agar base (Himedia, India) which was enriched with 5% sheep blood, and MacConkey agar (Himedia, India). Bacterial growths were identified and recorded after incubation for 24 to 48 hours at 37°C aerobically. Identification of bacterial isolates was done based on colony morphological features and hemolytic reactions (primary cultures), gram staining reactions and biochemical tests (INVIC, Catalase and Coagulase tests) on pure cultures (Quinn *et al*., 2002).

Questionnaire survey

During farm visits, a semi-structured questionnaire was used to collect data about herd and cow level variables thought to influence the prevalence of mastitis. The questionnaire was administered to farm owners/attendants through a face-to-face interview by four final year undergraduate veterinary students (one in each site) who were conducting research for graduation. Some of the variables were recorded by direct observation of the milking and husbandry practices. The data collectors had received training before initiation of the research to ensure that recording was consistent. The herd level variables recorded were herd size, management (intensive or semi-intensive), floor type (concrete, wood or soil), bedding (yes or no), pre or post milking teat dipping, udder washing practices (whole udder or teats only), housing (stall barn or group barn), use of towel for drying (yes or no), whether mastitic cows milked last or not, culling chronically infected cows, and dry cow therapy. Cow level data included age, parity, stage of lactation, udder position (normal or pendulous), teat end shape (pointed, round or flat), cow dirtiness (clean, slightly dirty, moderately dirty or very dirty), and previous history of mastitis.

Data analysis

Data collected through questionnaire survey and CMT were entered into Microsoft Excel spread sheet and then exported to Stata 14.2 statistical software (StataCorp, 4905 Lakeway Drive, College Station, Texas) for analysis. The prevalence of cow-level mastitis was calculated by dividing the number of mastitis-positive cows (clinical and subclinical) by the total number of animals tested while herd-level prevalence was determined by dividing positive herds by total number of herds. A herd was considered as positive if at least one cow tested positive for clinical or subclinical mastitis. Possible risk factors for mastitis were selected using univariable mixed effect logistic regression analysis with farm ID as a random effect to account for clustering at herd level. All variables having p-value <0.25 in the initial univariable analysis were further checked for co-linearity using Kruskal gamma statistics before multivariable analysis, and those variables whose gamma value ranged between −0.6 and +0.6 were considered in a multivariable logistic regression model. During multivariable mixed effect logistic regression analysis, all non-significant variables were removed sequentially by backward elimination where the model with the lowest Akaike Information Criterion (AIC) value was chosen as the best model. At every step during model development, the confounding effect of herd size was assessed by checking for changes in parameter estimates, and changes >25% were considered to indicate confounding (Dohoo *et al*., 2009). To compare differences in cow level prevalence in the four sampling towns, logistic regression with sampling town as a categorical variable and a farm as a random effect was performed. In all analyses, confidence levels were calculated at 95% and a p value < 0.05 was used for statistical significance level.

Results

The overall prevalence of mastitis at cow level was 54.2% (95% CI: 50.5 – 57.9%). The majority of mastitis cases in the cows tested was subclinical (48.1%) while the prevalence of clinical mastitis was only 6.1%. No significant ($p > 0.05$) difference was noted in the prevalence of mastitis across the four sampling towns (Table 1). Among the 122 herds examined in the study, 109 (89.3%; 95% CI: 82.1 – 93.9%) of them had at least a cow positive for mastitis. All the herds examined in Wolayta Sodo, 95.8% in Arsi Negele, 85.4% in Hawassa and 83.3% in Wendo Genet were positive for mastitis. The prevalence varied between 0% and 100% within a herd at an average herd prevalence of 52% (Table 2).

Table 1. Prevalence of cow-level clinical and subclinical mastitis in dairy cows in the southern Ethiopia

Sampling town	No examined	Clinical mastitis	Subclinical mastitis	Overall	95% CI
		Positive (%)	Positive (%)	Positive (%)	
Wendo Genet	135	17 (12.6)	54 (40)	71 (52.6)	44.1 – 60.1
Wolayita Sodo	152	4 (2.6)	76 (50)	80 (52.6)	44.7 – 60.5
Arsi Negele	94	10 (10.6)	41 (43.6)	51 (54.3)	44.1 – 64.1
Hawassa	305	11 (3.6)	159 (52.1)	170 (55.7)	50.1 – 61.2
Total	**686**	42 (6.1)	330 (48.1)	**372 (54.2)**	**50.5 – 57.9**

Table 2. Herd-level prevalence of mastitis in dairy farms in the southern Ethiopia

Sampling town	Herds tested	Positive herds	Prevalence (%)	95% CI
Wendo Genet	36	30	83.3	66.5 – 93.0
Wolayita Sodo	21	21	100	80.8 – 100
Arsi Negele	24	23	95.8	76.8 – 99.8
Hawassa	41	35	85.4	70.2 – 93.9
Overall	122	109	89.3	82.1 – 93.9

Out of the 2744 quarters examined, 111 (4.1%) were found blind and nonfunctional. The frequency of blind teats was slightly higher on the hind quarters than front quarters, and the overall quarter level prevalence of mastitis was 29.4% (Table 3).

Table 3. Proportion of blind quarters and prevalence of mastitis at quarter level in dairy cows in southern Ethiopia

Quarter	No quarters	Blind quarters, n (%)	No quarters tested	No positive	Prevalence (%)
RH	**686**	**35 (5.1)**	**651**	**184**	**28.3**
LH	686	24 (3.5)	662	203	30.7
RF	686	29 (4.2)	657	195	29.7
LF	686	23 (3.4)	663	191	28.8
Total	**2,744**	**111 (4.1)**	**2,633**	**773**	**29.4**

RF = Right front; LF = Left front; RH = Right hind; LH = Left hind;

In the present study, various possible risk factors at cow and farm levels were evaluated for their effect on mastitis prevalence. Univariable mixed effect logistic regression was performed on age, parity, lactation stage, udder position, teat end shape, history of mastitis, cow dirtiness, herd size, milking mastitic cow, management (intensive/semi-intensive), floor type (concrete, wood or soil), bedding (yes or no), washing udder before milking (whole udder or teats only), housing (stall barn or group barn), use of towel for drying (no, separate or common) and sampling town (Table 4). Out of 16 possible risk factors analyzed, parity, lactation stage, udder position, teat end shape, history of mastitis, cow dirtiness, milking mastitic cow and herd size had $p < 0.25$ and thus selected for multivariable analysis. Co-linearity was checked between variables before multivariable analyses. Accordingly, age was dropped from further analysis due to co-linearity with parity (gamma = 1) and history of mastitis in preceding lactation (gamma = 0.64). Parity was retained in the analysis due to its higher biological importance than age in relation to mastitis. The best fit model included parity, teat end shape, cow dirtiness, and history of mastitis as significant factors associated with prevalence of mastitis in cows. The final model showed that higher parity (OR = 1.6; $p = 0.018$), history of mastitis in preceding lactation (OR = 3.4; $p < 0.001$), round (OR = 2.2; $p < 0.001$) and flat teat ends (OR = 4.6; $p < 0.001$), and slightly dirty (OR = 2.9; $p < 0.001$), moderately dirty (OR = 3.2; $p < 0.001$) and very dirty (OR = 8; $p = 0.001$) udder and legs were significant factors associated with cow-level mastitis prevalence (Table 5). Herd size showed no association with mastitis prevalence ($p = 0.109$). However, the role of herd size as a confounder was investigated by fitting models for mastitis prevalence with and without herd size included. None of the coefficients for the other variables changed substantially when herd size was excluded, so we concluded that any confounding effect of herd size was minimal.

Table 4. Results of univariable mixed effect logistic regression analysis of potential animal and herd level risk factors with mastitis prevalence

Risk factors	Category	No cows examined	No cows positive (%)	Crude OR (95% CI)	p-value
Age	≤ 8yrs	576	289 (50.2)	1	
	> 8yrs	110	83 (75.5)	3.1 (1.9 – 4.9)	<0.001
Parity	Primiparous	177	69 (40)	1	
	Multiparous	509	303 (59.5)	2.4 (1.6 – 3.4)	<0.001
Lactation	≤ 4 month	209	102 (48.8)	1	
	> 4 to 8 month	335	185 (55.2)	1.3 (0.9 – 1.9)	0.127
	> 8 month	142	85 (59.9)	1.6 (1.0 – 2.5)	0.038
Udder position	Normal	569	287 (50.4)	1	
	Pendulous	117	85 (72.7)	2.7 (1.7 – 4.2)	<0.001
Teat end shape	Pointed	288	117 (40.6)	1	
	Round	331	199 (60.1)	2.5 (1.7 – 3.6)	<0.001
	Flat	67	56 (83.6)	8.1 (3.9 – 16.7)	<0.001
History of mastitis	No	458	196 (42.6)	1	
	Yes	228	176 (77.2)	4.7 (3.2 – 6.9)	<0.001
Cow dirtiness	Clean	229	81 (35.4)	1	
	Slightly dirty	285	176 (61.8)	3.5 (2.3 – 5.4)	<0.001
	Moderately dirty	149	97 (65.1)	4.9 (2.8 – 8.7)	<0.001
	Very dirty	23	18 (78.3)	9.2 (2.9 – 29.2)	<0.001
Herd size	≤ 10	162	78 (48.2)	1	
	> 10	524	294 (56.1)	1.4 (0.9 – 2.0)	0.109
Management	Semi-intensive	105	58 (55.2)	1	
	Intensive	581	314 (54)	0.97 (0.6 – 1.7)	0.920
Floor type	Wood	17	6 (35.3)	1	
	Earth/soil	37	19 (51.4)	0.5 (0.1 – 1.95)	0.336
	Concrete	632	347 (54.9)	1.1 (0.5 – 2.4)	0.750
Housing	Stall barn	214	117 (54.7)	1	
	Group barn	472	255 (54)	0.95 (0.6 – 1.4)	0.803
Bedding	Yes	136	72 (52.9)	1	
	No	550	300 (54.6)	1.0 (0.7 – 1.6)	0.927
Udder washing before milking	Teats only	46	22 (47.8)	1	
	Whole udder	635	348 (54.8)	0.76 (0.4 – 1.5)	0.430
Towel use	No	152	75 (49.3)	1	
	Common	142	76 (53.5)	1.3 (0.8 – 2.0)	0.244
	Separate	392	221 (56.4)	1.2 (0.7 – 1.97)	0.515
Milking mastitis cow last	No	270	139 (51.5)	1	
	Yes	416	233 (56)	0.8 (0.6 – 1.2)	0.268

CI = Confidence interval; OD=Odds ratio

Table 5. Best-fit multivariable model for risk factors associated with cow-level mastitis prevalence using mixed effect logistic regression modelling with farm as random effect

Risk factors	Category	Crude OR (95% CI)	Adjusted OR (95% CI)	p-value
Parity	Primiparous	1	1	
	Multiparous	2.3 (1.6 – 3.3)	1.6 (1.1 – 2.5)	0.018
History of mastitis	No	1	1	
	Yes	4.5 (3.2 – 6.5)	3.4 (2.3 – 5.1)	<0.001
Teat end shape	Pointed	1	1	
	Round	2.2 (1.6 – 3.0)	2.2 (1.5 – 3.2)	<0.001
	Flat	7.4 (3.7 – 14.8)	4.6 (2.1 – 9.9)	<0.001
Cow dirtiness	Clean	1	1	
	Slightly dirty	2.9 (2.1 – 4.2)	2.9 (1.9 – 4.4)	<0.001
	Moderately dirty	3.4 (2.2 – 5.3)	3.2 (1.8 – 5.6)	<0.001
	Very dirty	6.6 (2.4 – 18.4)	8.0 (2.4 – 26.1)	0.001
Constant			0.1 (0.04 – 0.21)	<0.001

CI = Confidence interval; OD=Odds ratio

Bacteria isolated

Bacterial examination was performed on 307 milk samples collected from cows with subclinical mastitis. However, due to lack of media and other facilities, isolation was limited to only certain bacteria. Accordingly, growth of different types of bacteria was observed in 299 (97.4%) of the samples cultured. *Staphylococcus* spp. were the most prevalent bacteria isolated from 57.3% of the samples cultured. *Streptococcus* spp., *E. coli* and *Bacillus* spp. were the other bacteria isolated in decreasing order of their prevalence (Table 6).

Table 6. Bacteria isolated from mastitic milk samples (N = 307)

Type of bacteria	No isolates	Prevalence (%)
Staphylococcus spp.	176	57.3
Streptococcus spp.	57	18.6
E. coli	53	17.3
Bacillus spp.	23	7.5

Discussion

Mastitis is the most costly disease in the dairy industry worldwide. It is a complex disease that results from the interaction of many factors involving the host, agent and environment. This study investigated the prevalence, risk factors and certain bacterial causes of mastitis in four towns of southern Ethiopia known for their dairy cattle potential. Unlike most of the previous studies in the region, the information presented in this paper was derived from a large sample of 686 lactating cows found in 122 dairy farms. An overall cow-level prevalence of 54.2% and overall herd-level prevalence of 89.3% was recorded. The cow-level prevalence of this study is comparable to two previous studies in Ethiopia: 52.9% in and around Areka town (G/Michael *et al*., 2013) and 56.5% in Batu and its environs (Duro and Taddele, 2011). However, it is higher than those reported in other studies that used CMT viz. 23.18% in Doba district, West Hararghe Zone (Girma *et al*., 2012), 39.5% in Adama town (Belayneh *et al*., 2013), 46.9% in and around Gondar town (Alemu *et al*., 2013) and 40.1% in Bishoftu town (Birhanu *et al*., 2017). In contrast, the present prevalence is lower than a previous report of 66.6% in Asella (Abera *et al*., 2013), 64.3 % in Adigrat (Zenebe *et al*., 2014), 62.6 % in Hawassa milk shed (Abebe *et al*., 2016) and 62% in North-West Ethiopia (Mekonnen *et al*., 2017). The first meta-analysis of the prevalence of mastitis in dairy cattle in Ethiopia conducted by Getaneh and Gebremedhin (2017) indicated that the variation of mastitis prevalence between studies might be due to variation in the locality and period of study, number of animals sampled, breeds, stages of lactation, parity number, and management practices.

In the present study, higher prevalence of subclinical mastitis (SCM) (48.1%) was observed compared to the clinical form (6.1%), a finding that is in line with previous studies (Debele, 2010; Moges *et al*., 2011; Abebe *et al*., 2016; Kebebew and Jorga, 2016). Risk factors including higher number of parity, history of mastitis in preceding lactation, flat or round teat end shapes in cows, and cow dirtiness were the likely attributable factors to the observed high prevalence of SCM. Furthermore, absence of mastitis prevention and control practices such as post milking teat disinfection, culling chronically infected cows and dry cow therapy by most of the dairy farms are the other possible reasons. Due to lack of mastitis monitoring program and absence of visible clinical signs, cows infected with SCM remain undetected for a long time. This increases the probability of mastitis transmission from infected to uninfected cows within herds by the hands of milker's without the notice of the farmers (Radostits *et al*., 2007).

The odds of mastitis was 1.6 times higher in multiparous than primiparous cows. This finding is consistent with several previous studies (Abunna et al., 2013; Belayneh et al., 2013; Katsande et al., 2013; Zeryehun et al., 2013; Abrahmsén et al., 2014; Abebe et al., 2016). Higher prevalence of mastitis in multiparous cows might be ascribed to loosening of sphincter and patency of teat canal in older cows. Moreover, the median ligaments, which provide support to the teat, also get relaxed with age leading to hanging of udder and thus it makes more prone to mastitis (Boujenane et al., 2015; Bhat et al., 2017).

In the present study, cows with flat teat ends (OR = 4.5) and round teat ends (OR = 2) were more likely to have mastitis compared to those with pointed teat ends. This is perhaps because flat or round teats have wider streak canals, which can give greater chance for the entry of infectious agents, than pointed teat ends (Appleman, 1970). A similar finding was also reported by other studies like Belayneh et al. (2013), Nakov et al. (2014) and Abebe et al. (2016).

Cows with a history of mastitis in the preceding lactation were 3.3 times more likely to have mastitis than those without mastitis history. This finding is in agreement with previous studies (Houben *et al*., 1993; Berry and Meaney, 2005; Abebe *et al*., 2016). According to Elmaghraby et al. (2017), the reason for recurrent mastitis can be a persistent infection of the mammary gland by a mastitis pathogen.

The odds of finding a cow with mastitis increased as the degree of cow dirtiness increased. It was noted that the likelihood of mastitis was 8, 3.2 and 2.9 times higher in cows with very dirty, moderately dirty and slightly dirty udder and legs as compared to those with relatively clean udder and legs, respectively. It is obvious that the dirtiness of udder and hind legs is the result of poor hygiene of the cow's environment and facilities in the cows' barn. As stated by Rajabi *et al*. (2017), poor cow hygiene can contribute to presence of mastitis pathogens on teat ends and increasing the rate of new infections. Similar to the current finding, other researchers have also reported a significant association between mastitis prevalence and poor udder and leg hygiene (Abrahmsén *et al*., 2014; Iraguha *et al*., 2015; Abebe *et al*., 2016; Mureithi and Njuguna, 2016)

Various types of contagious and environmental pathogens have been reported to cause bovine mastitis in Ethiopia. However, only four types of bacteria were isolated in the present bacteriological study due to lack of the required media and reagents. *Staphylococcus* spp. were the most dominant organisms isolated followed by *Streptococcus* spp. As stated by Mdegela et al. (2009), the dominance of *Staphylococcus* spp. in bovine mastitis is possibly a result of poor milking hygiene. The predominance of *Staphylococcus* spp. particularly *S. aureus* has also been reported by several bovine mastitis studies in Ethiopia (Mekibib *et al.,* 2010; Abera *et al*., 2013; Belayneh *et al*., 2013; Zeryehun *et al*., 2013).

Conclusions

This study demonstrated a high prevalence of mastitis. Subclinical mastitis was the major form prevalent among the dairy farms in southern Ethiopia. Higher number of parity, history of mastitis in the preceding lactation, teat end shapes and cow dirtiness were important risk factors of bovine mastitis in the study area. The study also showed *Staphylococcus* spp., *Streptococcus* spp., *E. coli* and *Bacillus* spp. as possible causes of mastitis in the dairy farms. Therefore, raising awareness of dairy farmers, making the animals' environment clean and dry as possible, post milking teat dipping and regular screening and culling chronically infected cows are recommended as feasible interventions.

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