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INCIDENCE OF DAIRY COW MASTITIS AND ASSOCIATED RISK FACTORS IN SODO TOWN AND ITS SURROUNDINGS, WOLAITA ZONE, ETHIOPIA

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ABSTRACT

A longitudinal observational study on the incidence of mastitis in smallholder dairy cows in Sodo town and its surroundings, Ethiopia was conducted during the period from October 2015 to March 2016 with the aim of estimating incidence of mastitis in smallholder dairy cows, investigating potential risk factors associated with mastitis, and isolating mastitis-causing bacteria in milk of smallholder dairy cows. All the sixty-seven lactating cows from the nine smallholder dairy farms were registered for the follow up study. The incidence of mastitis at cow and quarter levels was followed up for six months. On top of that, a questionnaire survey on smallholder dairy cow management and milking procedure was performed at the farms where the study animals resided. The results of this study revealed 50.7 % (n = 34/67) prevalence of mastitis at cow and 29.1 % (n = 78/234) at quarter levels. The total incidence risk was found to be 60.6 % (n = 20/33). Based on bacteriological examination, 90.8 % of the collected samples (n = 59/65) were found to be mastitis bacteria positive. Pathogenic bacteria belonging to five genera were involved in causing mastitis. Among these isolates, *Staphylococcus aureus* was the predominant mastitis pathogen (n = 23/65, 39 %) found in the study area followed by *Streptococci* species (n = 12/65, 20.3 %), Coagulase-negative *Staphylococci* species (n = 11/65, 18.6 %), *Escherichia coli* (n = 8/65, 13.6 %), and *Bacillus* species (n = 3/65, 5.0 %). The least mastitis pathogen isolated was *Corynebacterium* spp. (n = 2/65, 3.4 %). The influence of 22 potential risk factors on the incidence of mastitis was also investigated. Among others, late lactation stage, low daily milk yield, male milkers, dry cow therapy only at last milking of lactation were elicited to be highly risky for mastitis. On the other hand, milkers' work experience, herd size, teat distance from the ground, and milkers' skill of finding milk clots appeared to be not risky for the incidence of mastitis whereas, age greater than or equal to eight year, parity above or equal to six, and milk yield less than three liters per day were significantly associated with the prevalence of mastitis ($p < 0.01$. $p < 0.05$, $p < 0.05$, respectively). Although not significant, owners as milkers and late lactation stage had higher influence on the prevalence of mastitis ($p = 0.058$ and $p = 0.147$, respectively). In conclusion, the relatively high incidence of mastitis in the study area can be responsible for serious impact on the economy of smallholder dairy farmers mainly by reducing the quantity and quality of milk yield and undermining fertility of the dairy cows. Thus, continuous education of the smallholder dairy farmers is needed for better mastitis control programs.

Key words: mastitis; dairy cows; risk factors; udder pathogen

INTRODUCTION

Causative agents of mastitis with zoonotic potential may represent a health risk for human populations via food chain (Bradley, 2002). Thus, extra attention should be paid to the study of mastitis.

Mastitis, inflammation of the parenchyma of mammary gland is a complex disease of dairy cows (Idriss *et al*., 2013). It is accompanied by physical, chemical, pathological and bacteriological changes in milk and glandular tissue (Samad, 2008). Almost any bacterial or mycotic organism that can opportunistically invade tissue and cause infection can cause mastitis. Over 135 different microorganisms have been isolated from bovine intra-mammary infections, but the majority of infections are caused by staphylococci, streptococci, and gram-negative

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bacteria (Bradley, 2002). Mastitis of dairy cows is often described as subclinical or clinical mastitis. Subclinical mastitis is the presence of an infection without apparent signs of local inflammation or systemic involvement. It is always related to low milk production, changes to milk consistency, reduced possibility of adequate milk processing, low protein and high risk for milk hygiene since it may even contain pathogenic organisms (Tancin *et al.*, 2007; Sharma *et al.*, 2011). Whilst what the farmer sees is clinical mastitis, subclinical mastitis is more serious and is responsible for much greater loss to the dairy industry (Kader *et al*., 2002). Cows with subclinical mastitis should be considered a risk of spreading mastitis pathogens within and between herds and are as such of a national concern (Person *et al*., 2011). It is, therefore, important to know the prevalence of subclinical mastitis in dairy herds and delineate the important factors responsible for it.

Detection of subclinical mastitis is best done by examination of milk for somatic cell counts using either the California Mastitis Test (CMT) or automated methods. The CMT is the most reliable and inexpensive cow side test for the detection of subclinical mastitis worldwide. It is an indirect measure of cell count. The CMT reagent contains a detergent that reacts with DNA of the cell nuclei, and a pH indicator (Bromo cresol purple) that changes the color when the milk pH increases above its normal value (Radostits *et al.*, 2007). Somatic cells are mainly milk-secreting epithelial cells that have been shed from the lining of the gland and white blood cells (mainly neutrophyls) that have entered the mammary gland in response to injury or infection. They are normal constituent of milk and only when they become excessive do they indicate intra-mammary infection (Reksen *et al*., 2008). Somatic cells are composed of leukocytes (75 %) and epithelial cells (25 %) (Henna Hamadani *et al.*, 2013). The leukocytes are attracted to the area of inflammation, where they attempt to fight the infection. In general, it is accepted that somatic cell count (SCC) is a golden standard in diagnostics of any form of mastitis in udder (Pyörälä, 2003).

In Sodo town and its surrounding, the number of smallholder dairy farms has a tendency to increase. However, the economic benefit they acquire from dairy farms is not inspiring. Although it is assumed that mastitis plays important role in the reduction of milk quantity and quality and poor fertility of dairy cows in the study area, limited studies have been conducted on the contribution of mastitis, especially subclinical mastitis, to the problem of these smallholder dairy herds and on their management practices related to mastitis problems. The study on the incidence of mastitis, mastitis causative bacteria, and factors associated with mastitis is thus important to design relevant mastitis prevention strategy. Therefore, this study was carried out with the objectives of estimating incidence of mastitis in smallholder dairy cows in Sodo town and its suburb, investigating factors associated with the incidence of mastitis, and isolating bacteria causing mastitis in milk of smallholder dairy cows.

MATERIAL AND METHODS

Study animals

A longitudinal observational study was conducted during the period from October 2015 to March 2016 to estimate incidence of mastitis in smallholder dairy cows. All the sixty-seven lactating cows that were found in nine smallholder dairy farms were registered for the follow up study. The study animals were managed under intensive farming system. The housing system was tie-stall. They were milked by hand twice a day at the tie stall. The animals were provided either with green grass or hay and/or crop residue according to the availability of the feed. They were supplemented with wheat bran. The animals were visited by investigators every month during the morning milking time for six consecutive months (on days 0, 30, 60, 90, 120, 150, and 180). During each visit, their mammary glands were first visually then physically examined for any pathological change and for the presence of blind teats.

Milk samples also collected from these dairy cows were immediately subjected to physical examination with naked eyes to detect any abnormalities in color, odor, consistency and presence of clot, blood, flakes and any other visible abnormalities. In addition to the physical examination of the milk, CMTs were performed at the farms to determine whether the dairy cow is positive or negative for mastitis. California Mastitis Test (CMT; Bovi-VetTM, Kruuse, Germany) was carried out on all quarters with the exception of quarters with blind teats. The milk samples were collected, after the quarters were washed and dried, the first few squirts of milk were discarded and about 3 ml of milk was collected from each quarter into the respective wells of the CMT paddle and an equal volume of CMT reagent was added into each well. The paddle was swirled to thoroughly mix the contents for about 20 seconds. According to the amount of gel formed, the reaction was visually scored according to the formation of reaction or not as “negative” and “positive”. On day zero visit, mastitis positive dairy cows were identified and excluded from the follow up study. The remaining animals were considered as mastitis at risk and followed up for the incidence of mastitis. Thereafter, at each visit of the smallholder dairy farms, mastitis positive cows were excluded from the follow up study. Further, management practices, housing conditions, and milking routine of each small holder dairy farm were observed during each visit. All examinations and data collection for this study were carried out by the investigators.

Milk sample collection for bacteriological isolation

The milk samples were taken shortly prior to milking and only cows with strongly CMT positive quarters were sampled. A total of 65 quarters from the nine smallholder farms were sampled for bacteriological isolation. The milk samples were collected by a standard milk sampling technique as described by Quinn *et al.* (2002). After a quarter had been cleaned up by removing any possible dirt and washed with water, the teat end was dried and swabbed with cotton soaked in 70 % ethyl alcohol. Approximately 10 ml of milk was collected aseptically into sterile bottles, after discarding the first three milking streams. Milk samples from each quarter were transported to Wolaita Zone Regional Laboratory in an ice-cooled box at 4 ºC and analyzed immediately for identification of the mastitis pathogen.

Media preparation

Both general purpose and selective media were prepared and used according to their manufacturers' guidelines. Measured amount of media was added to a flask containing known volume of distilled water and placed into hot plate stirrer until it boils. Then, bacteriological media were placed within the autoclave and sterilized at 121 ºC for 15 minutes. The medium was cooled to 45–50 ºC inside water bath and 5 % sterile sheep blood was added to blood agar base medium for the growth of fastidious *Streptococcus* species that require enriched media. Then, the media was poured into different size petri-plates under aseptic condition inside Bio-safety Cabinet (Bioair Instruments, Eurolone Company, Italy) and allowed some time to solidify. Similarly, the agar media was also poured into test tube and slants were made. On the other hand, broth media were prepared in a test tube inside bio-safety cabinet. After preparation, all bacteriological media were placed into an incubator adjusted to 37 ºC for overnight to check the growth of contaminant. Petri-plates and test tubes free from contaminant were used for culturing of milk sample.

Identification of bacteria

A loopful of each milk sample was inoculated on blood agar base enriched with 5 % defibrinated sheep blood (Oxoid Ltd, Basingstoke, Hampshire, England) (Wasilauskas *et al.*, 1974); and incubated for 18–24 hours at 37 °C. Then returned to the incubator for at least another 24–48 hours and reexamined for the presence of slow growing bacteria. Different colonies were sub-cultured and incubated again on blood agar base and MacConkey agar (Oxoid Ltd, Basingstoke, Hampshire, England) until clear separated colonies were observed in a petri-plate. Then, the pure colonies were transferred to nutrient agar (Oxoid Ltd, Basingstoke, Hampshire, England) and allowed to grow inside the incubator. The identification of bacteria were made using colony morphology, hemolytic characteristic; gram staining, catalase test, coagulase test, CAMP test and IMViC (Indole, Methyl red, Voges-Proskauer, Citrate) (Cheesbrough, 2006; Quinn *et al.*, 2002). Additionally, the isolation of microbes were made using selective and differential media like MacConkey agar, Bacillus cereus agar, Mannitol salt agar and purple base agar (Oxoid Ltd, Basingstoke, Hampshire, England), and Eosin methylene blue agar (Himedia, Mumbai, India).

Questionnaire survey

Data from each animal and herd were collected using an individual questionnaire. The purpose of the questionnaire survey was to gather information on the farm and its management practices in addition to each visit observation of the farm. Accordingly, parameters studied were age, breed, number of parity, lactation stage and per day milk production, milking procedure, milkers' experience and their sex, manure management in the farm. Age, parity, lactation stage were determined by asking owner and farm attendant as well as from the farm records where available.

Statistical analysis

The collected data were checked for any inconformity and inconsistence and entered into Excel spreadsheet, coded, and transferred to SPSS version 20. Both the questionnaire and CMT data were analyzed on the given statistical package software. For descriptive statistics presentation of categorical data, Chi-square was used to compare the different groups of age, sex, and various risk factors, with the outcome variable (mastitis). A p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 67 lactating dairy cows from nine smallholder dairy farms were examined for the presence of mastitis. Of these, 34 dairy cows (50.7 %) were found to be mastitis positive. All the milk samples collected from 234 quarters were subjected to CMTs. Seventy eight (29.1 %) of them showed positive reaction for mastitis. The prevalence of mastitis at cow level was highest in herds where small numbers of cows were lactating compared to herds with high number of lactating cows (Table 1). Twenty nine point four percent (n = 10) of the 34 mastitis positive dairy cows had one quarter infected, while 26.5 % (n = 9) had two quarters, 29.4 % (n = 10) three quarters, and 14.7 % (n = 5) four quarters affected (Table 2). The majority of blind teats originated from a farm where Jersey breed cows were reared (28.6 %, n = 32/112 quarters). In general, a total of 34

Table 1: Prevalence of mastitis at cow and quarter levels

Farm	No. of cows examined	No. of CMT positive cows	Cow level prevalence (%)	No. of quarters examined	CMT positive quarters	Quarter level prevalence (%)
F01	9	5	55.6	36	14	38.9
F02	9	5	55.6	36	16	44.4
F03	28	10	35.7	112	14	12.5
F04	3	1	33.3	12	2	16.7
F05	3	3	100.0	12	6	50.0
F06	3	3	100.0	12	6	50.0
F07	3	1	33.3	12	2	16.7
F08	4	3	75.0	16	10	62.5
F09	5	3	60.0	20	8	40.0
Total	67	34	50.7	268	78	29.1

Table 2: The proportion of quarter affected from mastitis positive cows

Farm	No. of quarter affected and prevalence									
	one	%	two	%	three	%	four	%	total (+) cows	%
F01	1	10.0	0	0.0	3	30.0	1	20.0	5	14.7
F02	0	0.0	1	11.1	2	20.0	2	40.0	5	14.7
F03	7	70.0	2	22.2	1	10.0	0	0.0	10	29.4
F04	0	0.0	1	11.1	0	0.0	0	0.0	1	2.9
F05	1	10.0	1	11.1	1	10.0	0	0.0	3	8.8
F06	1	10.0	1	11.1	1	10.0	0	0.0	3	8.8
F07	0	0.0	1	11.1	0	0.0	0	0.0	1	2.9
F08	0	0.0	1	11.1	0	0.0	2	40.0	3	8.8
F09	0	0.0	1	11.1	2	10.0	0	0.0	3	8.8
Total	10	29.4	9	26.5	10	29.4	5	14.7	34	100.0

Table 3: The prevalence of blind teat in different farms

Farm	No. of quarter examined	No. of blind teat	Prevalence (%)
F01	36	0	0.0
F02	36	2	5.6
F03	112	32	28.6
F04	12	0	0.0
F05	12	0	0.0
F06	12	0	0.0
F07	12	0	0.0
F08	16	0	0.0
F09	20	0	0.0
Total	268	34	12.7

Table 4: The incidence risk of mastitis in the study area

Farm	No. of animal initially at risk	No. of animal affected in the study period	Incidence risk (%)
F01	4	2	50.0
F02	4	4	100.0
F03	18	11	61.1
F04	2	1	50.0
F05	0	-	-
F06	0	-	-
F07	2	1	50.0
F08	1	1	100.0
F09	2	0	0.0
Total	33	20	60.6

Table 5: The incidence and relative risks of mastitis in association with animal risk factors

Risk factors		No. of animal initially at risk	No. of animals affected in the study period	Incidence risk (IR) (%)	Relative risk (RR)
Age (years)	3–5	16	10	62.5	1.1
	5–8	10	5	50.0	1.4
	≥ 8	7	5	71.4	
Parity	Primiparous	13	7	53.8	1.4
	2–5	16	10	62.5	1.2
	≥ 6	4	3	75.0	
Stage of lactation (months)	1–4	14	6	42.9	1.7
	4–7	3	2	66.7	1.1
	≥ 7	16	12	75.0	
Breed	Jersey	19	12	63.2	
	HF	12	7	58.3	1.1
	Cross	2	1	50.0	1.3
Milk yield (Lts)	< 3	8	8	100.0	
	3–8	16	9	56.3	1.8
	> 8	9	3	33.3	3.0
Teat distance from the ground	> 50 cm	15	9	60.0	
	< 50 cm	18	11	61.1	1.0

HF = Holstein Friesian

out of 268 quarters (12.7 %) were found to be blind in this study (Table 3).

The highest mastitis incidence risk was found in dairy farms, F02 and F08, which was 100 % (n = 4/4, n = 1/1, respectively) followed by F03 showing 61.1 % (n = 11/18) incidence risk of mastitis. Three of the remaining farms, F01, F04, and F07 had 50 % (n = 2/4, n = 1/2, n = 1/2, respectively). The total incidence risk of mastitis in the study area during the study period was found to be 60.6 % (n = 20/33) (Table 4).

Age group greater than eight years was 1.4 times more at risk for the incidence of mastitis than age group five to eight years and 1.1 times more at risk than age group three to five years. Animals on parity greater

than six were at higher risk to be affected by mastitis (1.4 times than primiparous), followed by dairy cows on two to five parity (1.2 times) and primiparous cows. Dairy cows at late stage of lactation were at higher risk of being affected by mastitis than animals at early and mid-lactation stages (1.7 times, 1.1 times, respectively). Jersey dairy cows were more at risk of mastitis, followed by Holstein Friesian, and Cross breeds (Exotic x Local) (Jersey dairy cows are 1.3 times at risk for mastitis than the cross breeds and 1.1 times than the Holstein Friesian cows). In this study, lowest milk production was found to be three times more at risk for mastitis than daily milk yield greater than eight liters and one point eight times than three to eight liter milk yield per day (Table 5). Management factors such as work experience of milkers, skill of finding milk clots in the milk streaks, and herd size were not associated with mastitis. Dairy farms that are secondary means of income for their owners are 1.3 times more at risk for mastitis than dairy farms that are the primary means of income. In addition, dairy cows that are hand-milked by male milkers are 1.5 times more at risk of acquiring mastitis than cows milked by female hand milkers. Dry cow therapy and removal of manure only once in a day were found to be 100 % risky for mastitis (Table 6). Washing udder and teat with flowing water was found to be 1.8 times more risky for mastitis than washing by soaking hand in a bucket of water. Examination of first streaks of milk was 1.3 times riskier for mastitis than not examining. Using common towel for drying udder before milking was found to be greater risk factor (1.5 times) for mastitis than using a new towel for each cow. In this study, milking dried udder and drying milker's hands before milking were shown to be higher risk factors for mastitis (1.2 times, 1.5 times, respectively) than milking wet udder and milking with wet hands of the milker. Similarly, washing hands after milking each cow was shown 1.2 times riskier for mastitis than not washing hands. Pre and Post-milking teat dipping were not practiced in the smallholder dairy farms (Table 7).

Prevalence of mastitis in dairy cows was

Table 6: The incidence and relative risks of mastitis in association with management

Variable	Description	No. of farms	No. of animal initially at risk	No. of animal affected	IR (%)	RR
As source of income	Primary	3	22	12	54.5	
	Secondary	6	11	8	72.7	1.3
Milker's sex	Male	4	26	17	65.4	1.5
	Female	5	7	3	42.9	
Experience of milker	≤ 5 years	3	5	3	60.0	
	> 5 years	6	28	17	60.7	1.0
Skill of identifying	Yes	6	27	17	63.0	1.3
sick udder	No	3	6	3	50.0	
Skill of finding milk clots	Yes	4	25	15	60.0	
Feeding cows	No	5	8	5	62.5	1.0
just after milking	Yes	4	10	7	70.0	1.2
Dry cow therapy	No	5	23	13	56.5	
	Yes	1	4	4	100.0	1.8
Regular surveillance	No	8	29	16	55.2	
of dry udder	Yes	2	6	5	83.3	1.5
Manure removal	No	7	27	15	55.6	
from the stall	Once/day	2	4	4	100.0	1.5
	> Once/day	7	29	16	55.2	
Herd size	≤ 10 animals	5	5	3	60.0	
	> 10 animals	4	28	17	60.7	1.0

IR = incidence risk, RR = relative risk

significantly ($p < 0.01$) associated with adult age of animals compared to young adult and young age (80.8 % vs 50.0 % vs. 17.4 %, respectively). Significantly more dairy cows ($p < 0.05$) at higher parity were found to be affected by mastitis than cows at 2–5 parity and primiparous (69.6 % vs 53.8 % vs 22.2 %, respectively). Low milk yield per day significantly ($p < 0.05$) influenced the prevalence of mastitis compared to relatively high and medium yielding dairy cows (83.3 % vs 52.0 % vs 36.7 %, respectively). Teat distance from the ground and lactation stages were not significantly ($p = 0.542$ and $p = 0.147$, respectively) associated with the prevalence

Table 7: The incidence and relative risks of mastitis in association with milking procedure

Variable	Description	No. of farms	No. of animal initially at risk	No. of animal affected	IR (%)	RR
Udder and teat washed by	Soaking hand in water	8	29	16	55.2	
	Flowing water	1	4	4	100.0	1.8
Milking with	Wet udder	2	4	2	50.0	
	Dry udder	7	29	18	62.1	1.2
Drying towel	Common	4	22	15	68.2	1.5
	Individual	5	11	5	45.5	
First streaks examination	Yes	4	27	17	63.0	1.3
	No	5	6	3	50.0	
Washing hands after milking each cow	Yes	3	10	7	70.0	1.2
	No	6	23	13	56.5	
Drying hands before milking	Yes	3	24	16	66.7	1.5
	No	6	9	4	44.4	

Table 8: Association of animal factors with prevalence of mastitis

Variable	Description	Mastitis prevalence	Total no. of animals	X^2	df	p-value
Age	3–5	4 (17.4 %)	23	19.6182	2	0.000
	5–8	9 (50.0 %)	18			
	≥ 8	21 (80.8 %)	26			
Parity	Primiparous	4 (22.2 %)	18	9.218	2	0.010
	2–5	14 (53.8 %)	26			
	≥ 6	16 (69.6 %)	23			
Lactation stage (months)	< 4	6 (33.3 %)	18	3.841	2	0.147
	4–7	6 (46.2 %)	13			
	≥ 7	22 (61.1 %)	36			
Milk yield	< 3	10 (83.3 %)	12	7.493	2	0.024
	3–8	11 (36.7 %)	30			
	≥ 8	13 (52.0 %)	25			
Teat distance from the ground (cm)	< 50	23 (76.7 %)	30	0.537	1.0	0.542
	≥ 50	31 (83.8 %)	37			

X^2 = Chi-square, df = degree of freedom, Significance = $p < 0.05$

of mastitis in dairy cows kept in smallholder farms. However, percentage of prevalence of mastitis was elevated with increased lactation stage compared to medium and early lactation stages (61.1 % vs 46.2 % vs 33.3 %, respectively) (Table 8). The highest prevalence of mastitis (p = 0.058) was found in lactating dairy cows milked by owners of the farm compared with cows milked by employee (100 % vs. 75.9 %). More dairy cows were affected by mastitis (p = 0.236) in smallholder dairy farms where the farm is used as secondary source of income than cows kept in farms that are used as primary source of income (87.5 % vs. 75 %). The percentage of dairy cows having mastitis was also higher (p = 0.342) in cows which were milked by different milkers every other day than in cows milked by the same milker (83.4 %, 73.1 %, respectively). Similarly, although not significant, increased prevalence of mastitis occurs in cows, which were milked by high school educated workers than in cows milked by primary school educated workers (87.1 %, 75 %, respectively). Further, results of this study revealed that prevalence of mastitis in cows was not influenced by milkers' work experience and frequency of manure removal (p = 0.514, p = 1.00, respectively) (Table 9).

The results of bacteriological culture on the milk samples collected from mastitis dairy cows considering only strong CMT positive samples are indicated in Table 10. 90.8 % of the collected samples (n = 59/65 samples)

Table 9: Association of farm management practices with prevalence of mastitis

Variable	Description (no. of farm)	Mastitis prevalence (%)	Total no. of animals	X^2	df	Significance
Farm as source of income	Primary (3)	27 (75.0)	36			
	Secondary (6)	27 (87.5)	31	1.559	1.0	0.236
Milker's work status	Employee (5)	41 (75.9)	54			
	Owner (4)	13 (100)	13	3.883	1.0	0.058
A cow is milked by	The same milker (4)	19 (73.1)	26			
	Different milker (5)	35 (83.4)	41	1.536	1.0	0.342
Milker's work experience	> 5 (7)	43 (79.6)	54			
	≤ 5 (2)	11 (84.6)	13	0.167	1.0	0.514
Milker's educational status	High school (6)	27 (87.1)	31	1.559	1.0	0.236
	Primary (3)	27 (75.0)	36			
Frequency of manure removal	Once/day	10 (83.3)	12	0.070	1.0	1.000
	> Once/day	44 (80.0)	55			

X^2 = Chi-square, df = degree of freedom, Significance = $p < 0.05$

Table 10: Bacteria isolated from mastitis dairy cows

Bacterial isolate	Frequency of isolation	Percentage of isolation (%)
Saphylococcus. aureus	23	39.0 %
Coagulase–Negative *Staphylococcus* spp	11	18.6 %
Streptococcus species	12	20.3 %
Bacillus species	3	5.0 %
E. coli	8	13.6 %
Corynaebacterium species	2	3.4 %
Total	59	90.8 %
No growth	6	9.2 %

were found to be mastitis bacteria positive whereas 9.2 % of the examined samples were negative (n = 6/65). Bacteria belonging to five genera were involved in causing mastitis. Both contagious and environmental bacteria were isolated. *Staphylococcus aureus* was the predominant mastitis pathogen (39 %, n = 23/65) found in the study area followed by *Streptococcus* species (20.3 %, n = 12/65), Coagulase–negative *Staphylococcus* species (18.6 %, n = 11/65), *Escherichia coli* (13.6 %, n = 8/65), and *Bacillus* species (5.0 %, n = 3/65). The least mastitis pathogen isolated was *Corynebacterium* spp. (3.4 %, 2/65).

DISCUSSION

The prevalence of mastitis at cow level in this study (50.7 %, n = 54/67) was almost similar to the findings (53.30 %) of Rahman *et al.* (2010) in Bangladesh, Bradley *et al.* (2007) (47.0 %) in Great Britain, Hashemi *et al.* (2011) (44.7 %) in Iran. It was higher than the results reported by Belayneh *et al.* (2013) (39.5 %) in Central Ethiopia, Girma *et al*. (2012) (23.18 %) in Eastern part of Ethiopia, and Abdel-Rady and Mohammed Sayed (2009) (19.14 %) in Egypt. On the other hand, it was less than the prevalence recorded by Yien Deng *et al.* (2015) (60.33 %) in Western Ethiopia and Muhamed Mubarak *et al*. (2012) (66.0 %) in India. The prevalence of dairy cows' mastitis in the Central and Eastern part of the country was lower than the prevalence in this study area (Southern part of the country) probably because the studies in those areas were conducted on cross and native breed dairy cows, respectively. Native and cross breed dairy cows are more resistant to mastitis compared to exotic breeds, which constituted above 90 % of the study animals in this study. The quarter level prevalence of mastitis (29.4 %) in the study area was comparable with the finding of Belayneh *et al.* (2013) and Hashemi *et al.* (2011) (23.7 % and 21.6 %, respectively) while it was much lower than the prevalence reported by Person *et al.* (2011), Muhamed Mubarak *et al*. (2012), Idriss *et al*. (2013), and Yien Deng *et al.* (2015) (60.0 %, 66.0 %, 73.85 %, and 47.21 %, respectively). This study revealed that 12.7 % of the quarters were blind (n = 34/268), which was a lot higher than the results reported by Girma *et al*. (2012) and Yien Deng *et al.* (2015) (2.2 %, n = 34/384 and 0.21 %, n = 1/484, respectively). Most of the blind teats (94.1 %) occurred in Jersey breed dairy cows (F03). This might be due to relatively wider teat openings and shorter teat distance from the ground; the teats are highly exposed to injury and thereby to blindness. Another finding of this study, which claimed that the Jersey breed dairy cows were 1.3 times more likely to develop mastitis than cross breed cows and 1.1 times more at risk than Holstein Friesian cows (Table 5) supports the above result.

According to the results of our study, the risks and prevalence of mastitis increased with advancing age, parity and lactation stage. Eight year and older dairy cows were 1.4 times at risk for mastitis compared to young adults (5–8 years old) and 1.1 time more at risk than young cows (3–5 years old). Similarly, dairy cows in parity number six and above were 1.4 times more at risk for mastitis than primiparous and 1.2 times more at risk than cows in parity number two to five. Correspondingly, Chi-square analyses revealed that prevalence of mastitis was significantly higher in adult (≥ 8 year old), cows at greater or equal to six parity, and cows yielded less than three liters of milk per day ($p < 0.01$, $p < 0.05$, and $p < 0.05$, respectively). These observations support the results recorded by Kerro and Tareke (2003), Islam *et al.* (2010), Girma *et al.* (2012), and Tancin (2013). It is possible to postulate that older cows have increased susceptibility due to depressed host defense mechanism. Age of cows approximates with parity. High risk of acquiring mastitis and prevalence of mastitis ($p = 0.147$) in dairy cows occurred in late lactation stage probably because of inefficient immune system response due to gradual change of feed formulation to dry cow diet, and stress triggered by advancing gestation. In this study, the greatest risk of developing mastitis and significantly higher ($p < 0.05$) prevalence of mastitis happened in daily low milk yielder cows (< 3 liters of milk.day^{-1}). Milk yield decreases as the lactation progresses. This coincided with the result reported by Du Preez (2000) where the somatic cell count usually increased only after the milk production of the cow failed to less than 4 kg per day. According to Radostits *et al.* (2007), prevalence of mastitis increases as the stage of lactation progresses. Research also showed that cows milked intermittently towards the end of lactation have dramatically increased somatic cell count (Blowey and Edmondson, 2000). This finding, therefore, asserted the aforementioned finding of the study; "there is high risk of acquiring mastitis in late lactation stage". Teat distance from the ground, milkers' skill of physical examination of milk, milkers' work experience, and herd size did not influence the incidence of mastitis in smallholder dairy farms in this study.

Smallholder dairy farms which were secondary means of income for the owners were more at risk for mastitis than farms that were primary source of income. Similarly, prevalence of mastitis was almost significantly high ($p = 0.058$) in smallholder dairy farms, where owners milk dairy cows (Table 9). 88 % of the surveyed smallholder dairy farms served as supplementary source of income to the owners. The smallholder dairy farmers operated with a very small resource base and earned much less than they required for livelihood from the farm. Thus, they cannot employ workers for the farm work.

In addition to milking and caring for thr animals, they have to spend much of their time working in other places to fill the gap in their costs of living. This probably partially diverted their attention that should have been fully paid to mastitis control and prevention. Dairy cows that were hand-milked by male milkers were highly (1.5 times more) at risk of acquiring mastitis compared to cows hand-milked by female workers. This might be because a woman knows better how to take care of dairy cows and handle proper hygienic conditions in the milking process. In this study, feeding cows just after milking and dry cow therapy just after the last milking of lactation were unexpectedly found to be highly risky for incidence of mastitis than cows not fed after milking and not treated during the early part of the dry period. Short acting antibiotics (Quick-release antibiotics) were used for dry cow therapy in the study area (Personal observation). Thus, the antibiotics remained active for a short period of time and probably protected the udder health in early dry period and thereafter intra-mammary infection might have occurred during the remaining dry period. This is supported by pioneer finding of Smith *et al.* (1985) where dry cow therapy was not effective during the pre-partum period. In addition, observational studies have shown that most infections with coliform and environmental streptococci take place in the last two weeks before calving (https://ahdc.vet.cornell.edu/programs/NYSCHAP/docs/). Peterson-Wolfe *et al.* (2010) also confirmed that cows are most susceptible to mastitis pathogens in the last seven to ten days of the dry period. Further, dry-cow therapy should be applied in conjunction with other mastitis control measures. Therefore, early dry and pre-partum periods can generally be considered critical for udder health. Based on this, it is safe to infer that in order to make dry cow therapy successfully effective in preventing udder infections to minimize the incidence of mastitis and ensure a production of safe for consumption milk, early dry period therapy should be repeated at two weeks pre-partum.

According to Jones (2006) and Idriss *et al.* (2013), the teat canals may remain partially open for 1–2 hour after milking. Hence, feeding cows just after milking is important to make animals remain standing to prevent pathogens from freely entering through the open teat canal. However, in this study a result opposite to the expected was documented. The explanation to this finding could be that the animals might be offered neither quality nor adequate amount of feed that could make them stand for 1–2 hours. Further, udder and teat washing with flowing water, milking with dry udder, first strips examination, washing hands after milking each cow and drying hands before milking unexpectedly failed to positively influence incidence of mastitis in the studied smallholder dairy farms. Most of the smallholder dairy farms in the study area were using unsanitized hand-borewell or river water for any purpose in the farms. They were also using common cloth towels for drying cows (Table 7) According to Peterson-Wolfe (2010), water should not be used as part of any milking procedure even if a sanitizing solution is added. According to him, sanitizers do not maintain activity throughout a milking, and water can introduce pathogens that are very difficult to cure. Using unsanitized hand-borewell or river water in the milking procedure probably had detrimental effect on unexpected results obtained in this study regarding to the incidence of mastitis. On top of using the unsanitized water, milkers' hand washing and udder washing were not carried out in accordance to valid norms. During udder washing, teat ends around the orifice were usually overlooked. The remaining dirt around the teat orifice might harbor mastitis-causing pathogens, which might freely enter into the teat canal during milking and cause intra-mammary infection. The milker's hands were simply rinsed with water only. Forestripping sub-clinically infected dairy cows and not properly washing hands might have served as means of transmission of mastitis to uninfected cows in the study area. Henna Hamadani *et al.* (2013) declared that the milker's hands should be washed thoroughly with disinfecting soap before milking. To further to prevent mastitis, Jones (2006) suggested approaching the milking procedure in the same way a surgeon approaches surgery: wash hands with soap and water, wash teats and udder in sanitizing solution, thoroughly dry teats and udder with individual towels, dip teats in an effective germicidal teat dip. Moreover, using common cloth towel to dry wet udder and teats of different cows might spread pathogens from sick/reservoir animal to other cows in the studied herds. As Henna Hamadani *et al.* (2013) reported, mastitis pathogens spread rapidly from cow to cow in the absence of pre and post-milking teat dipping. In these animals, transmission of mastitis infections can also occur through flies, especially by *Hydrotaea irritans* (Vasil, 2009).

90.8 % of the collected samples (n = 59/65 samples) were found to be mastitis bacteria positive. Whereas, 9.2 % (n = 6/65) of the strongly CMT positive collected milk samples were found to be mastitis bacteria negative. Bacteria-negative samples may occur due to spontaneous bacterial cure, the presence of too few viable bacteria for culture techniques, or death of the bacteria after removal of the milk sample from the gland but prior to culture (Zorah *et al*., 1993). Of the isolated bacteria, *Staphylococcus aureus* was the predominant mastitis pathogen (n = 23/59, 39 %) found in the study area followed by *Streptococcus* species (n = 12/59, 20.3 %), Coagulase–Negative *Staphylococcus* species (n = 11/59, 18.6 %), *Escherichia coli* (n = 8/59, 13.6 %), and *Bacillus* species (n = 3/59, 5.1 %). The least mastitis pathogen isolated was *Corynebacterium* spp. (n = 2/59, 3.4 %). Similarly, *Staphylococcus aureus* was the principal pathogen in Czech Republic, Denmark, and Germany

(Rysanek *et al.*, 2007; Schwarz *et al.*, 2010; Mohammed *et al.*, 2013). The prevalence of *E.coli* and *Bacillus* spp. in this study was close to the findings of Idriss *et al.* (2013) (12.3 % and 6.41 %, respectively), whereas the prevalence of *Staphylococcus aureus* in this study was much higher than claimed by Idriss *et al.* (2013) (9.74 % vs. 39 %). Among the bacterial culture isolates of this study, *Staphylococcus aureus* and *E.coli* belong to the most important major pathogens involved in bovine mastitis worldwide (Olde Riekerink *et al.*, 2008). *Staphylococcus aureus* is considered contagious (Barkema *et al.*, 1998) but environment *Staphylococcus aureus* mastitis may also occur (Zadoks *et al.*, 2002). *E. coli* is mainly of environmental origin (Munoz *et al.*, 2007). Other pathogens have both routes of infection (Idriss *et al.*, 2013). According to Sumathi *et al*. (2008), the relatively high incidence of environmental mastitis was due to poor hygiene of housing and milking conditions, as environment pathogens infect the udder through teat canal.

CONCLUSION

Incidence of mastitis at cow and quarter levels was found to be relatively high in the study area and can have serious economic impact on smallholder dairy farmers by reducing the quantity and quality of milk and undermining fertility of the dairy cows. The association of 22 potential risk factors with dairy cow mastitis was investigated. Of these factors, adult age, late lactation stage, low daily milk yield, male milkers, dry cow therapy only at last milking of lactation were elicited to be highly risky for mastitis. Similarly, adult age of dairy cows, increasing parity, and lower milk yield were significantly associated with the prevalence of mastitis. Using unsanitized hand-borewell or river water in the milking procedure, not washing hands and udder of dairy cows in accordance to valid norms, and using common cloth towel for drying udder and teats had detrimental effect on the incidence of relatively high mastitis. In order to minimize the incidence of mastitis, dry-cow therapy should be applied both at early dry period and at two weeks pre-partum in conjunction with other mastitis control measures.

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