

Prevalence, Risk Factors and Major Bacterial Causes of Bovine Mastitis in West Arsi Zone of Oromia Region, Southern Ethiopia

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Abstract: A cross-sectional study was conducted from October 2014 to April 2015 in Shashemene and Kofale, West Arsi zone of Oromia regional state with the objectives of determination of the prevalence of bovine mastitis, isolate the predominant bacterial agents involved in causing mastitis and identify associated risk factors. A total of 358 lactating cows (169 local and 189 cross) were examined for mastitis using clinical examination and California mastitis test (CMT). An overall prevalence of mastitis was recorded in the area 38% (136/358), of which 7.3% (26/358) were clinical and 30.7% (110/358) subclinical cases. Total animal examined in Shashamane was 165, out of these 35.8% (59/165) animals were positive for mastitis, from these animals, 8.5% (14/165) clinical and 27.3% (45/165) subclinical mastitis while 193 animals were examined from Kofale, out of these 39.9%(77/193) animals were positive for mastitis, from these animals, 6.2% (12/193) clinical and 33.7%(65/193) subclinical mastitis. Bacteriological methods were also employed to isolate the causative bacteria. About 83 bacterial isolates belonging to 7 species were identified from mastitic milk samples. The predominant isolated bacteria were *Staphylococcus aureus* (44.6%) followed by *Streptococcus agalactiae* (18.1%), *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were the least isolate which accounts 3.6% for each. There was no statically significant variation ($P>0.05$) between breeds, parity, age, but the prevalence of mastitis was found to be statistically significant among different hygiene of milking groups and lactation stages ($p<0.05$). The study also shows that mastitis is significant problem of dairy cows in the study area and the major isolated bacteria were contagious pathogens. The farmers should also be aware of the impact of the disease and practice hygienic milking, culling of chronic mastitis carriers and treating of clinically infected cows.

[Umer Seid, Tilahun Zenebe, Gizat Almaw, Abdela Edao, Haimanot Disassa, Tadele Kabeta, Firmaye Gerbi, Girma Kebede. **Prevalence, Risk Factors and Major Bacterial Causes of Bovine Mastitis in West Arsi Zone of Oromia Region, Southern Ethiopia.** *Nat Sci* 2015;13(8):19-27]. (ISSN: 1545-0740). <http://www.sciencepub.net/nature>. 4

Key words: Bovine, Mastitis, Major Pathogens, Prevalence, West Arsi Zone

1. Introduction

Ethiopia, located in tropical region, is one of the most populous countries in Africa, having an estimated population of more than 80 million. The country is very much dependent on agriculture. Livestock represent a major national resource and form an integral part of the agricultural production system. Ethiopia has the largest cattle population in Africa with an estimated population of 56.71 million. Cow represents the biggest portion of cattle population of the country, around 20.7% of the total cattle heads are milking cows (CSA, 2014). However, milk production often does not satisfy the country's requirements due to a multitude of factors. Mastitis is among the various factors contributing to reduced milk production (Biffa *et al.*, 2005). Dairy enterprise is very gradual in countries of sub Saharan Africa like Ethiopia. In this region, the low local milk production is as result of many factors including low genetic potential for milk production of indigenous breeds, the

extensive and low inputs husbandry practices under which they are reared and wide spread livestock diseases (Mohamed *et al.*, 2004).

Accordingly, few improved exotic breed animals that mostly limited in urban and peri-urban areas are yet not in position to satisfy the growing demand for milk of nation (Felleke and Geda, 2001). Conversely, low annual per capital consumption of milk in Ethiopia is 17-19 liters witnesses the gap between supply and basic demand of milk in urban areas (Vallezarate, 2000). Consequently, adequacy of the domestic milk production to cover the local demand has resulted in improving a considerable amount of dairy products. According to FAO (2003) in Ethiopia 42% of total cattle for private holdings are milking cows, however, milk production often doesn't satisfy the countries milk requirement due to multitude factors. There are several types of diseases which potentially infect and affect the wellbeing of livestock population among which mastitis is the common and

costly disease causing loss in milk yield, treatment cost for dairy farmers and culling of animals at unacceptable age (Vaarst and Envoldsen, 1997). It is considered as the most complex disease because of its multi factorial causation (Nibret, 2009).

Mastitis is an inflammation of the mammary gland and udder tissue, and is a major endemic disease of dairy cattle that follows a number of factors including the cow, the pathogen and the environment (Radostits *et al.*, 2007). It usually occurs as an immune response to bacterial invasion of the teat canal by variety of bacterial sources present on the farm, and can also occur as a result of chemical, mechanical and thermal injury to the udder. Mastitis as a disease has received little attention in Ethiopia, especially the subclinical form even though it is by far higher than clinical mastitis (Robertson, 1985). Efforts have only been concentrated on the treatment of clinical cases. In Ethiopia, the available information indicates that bovine mastitis is one of the most frequently encountered diseases of dairy cows (Stephen *et al.*, 2001). It is a major and prevalent disease of dairy cows causing huge economic loss as a result of reduced milk yield, early culling of productive cows (Fekadu, 1995) and a serious problem in the dairy industry of Ethiopia (Mekonnen *et al.*, 2005). The prevalence of clinical and sub clinical mastitis in different parts of Ethiopia is 1.2% and 21.5% respectively (Kassa *et al.*, 1999; Lemma *et al.*, 2001; Mungube, 2001; Kerro and Tareke, 2003; Werkinet *et al.*, 2002).

In Ethiopia, mastitis has long been known (Biffa *et al.*, 2005; Tamirat, 2007; Almaw *et al.*, 2009; Bitew *et al.*, 2010), however, the information on the magnitude, risk factors and causative agent of the disease is inadequate. Such information is important when designing appropriate strategies that would help to reduce its prevalence and effects (Biffa *et al.*, 2005). Most studies in Ethiopia were carried out in Addis Ababa and its surrounding, which are not representative of other regions of the country (Almaw *et al.*, 2009). In west Arsi zone, mastitis is not well considered. There is very few published material on the current status of mastitis in Shashaname (Abera *et al.*, 2012).

Hence this study was initiated with the objectives of:

- ✓ To estimate the prevalence of bovine mastitis in the selected districts of west Arsi zone,
- ✓ To assess the major risk factors associated with the occurrence of the bovine mastitis in the selected districts of west Arsi zone,
- ✓ To isolate and identify the major bacterial causative agents of bovine mastitis.

2. Materials and Methods

2.1. Study Area: The study was conducted in selected districts of West Arsi zone from October 2014 up to April 2015 in Kofale and Shashemene districts. Shashemene is located 250 km south of the capital Addis Ababa, and 25 km north of Awassa. The area lies within the Rift Valley, with altitudes ranging from 1700 to 2600 meters above sea level (m.a.s.l) and located at 7° 05'N to 7° 19'N and 38° 23'E to 38° 41'E. It receives an annual rainfall of 700–950 mm, and has an annual temperature range of 12–27°C. Out of the total area of 76,888 hectares, crop land accounts for 48,975 hectares, and the rest 7440, 5160, and 1320 hectares are forest land, grazing land and land for other purposes, respectively. The urban settlement accounts for 1733 hectares. The cattle population in the districts is 184,549 (SDARDO, 2010). While Kofale districts is located at 280 km south of Addis Ababa and located at 7° 19'N to 7° 40'N and 38° 30'E to 38° 53'E. Kofale is a highland, agro-pastoral area. Temperatures are moderate to hot. Some forests, including Arsi State forest, are found in Shashemene and Kofale districts. Rain fall is sufficient, which Kofale has erratic type of bimodal rainfall (KDARDO, 2010).

The human population of Shashemene districts is projected to be 100,454, directly living on agriculture and associated activities also by supplying its produce to the neighboring urban dwellers. While human population in Kofale districts is estimated around 178,950. The people of both districts belong to the Oromo ethnic community and others. Afan Oromo (the Oromo language) is the widely spoken language in the area (CSA, 2008).

2.2. Study Population: According to the recent census (CSA, 2014) Animal population of the west Arsi zone is 1,957,066 cattle, while 7.8% and 8.4% cattle population were found in Shashemene and Kofale, respectively. However, the study populations were all local and cross breeds of dairy cows that are managed under extensive, semi intensive and intensive farming system. The study was conducted on 358 local and cross breed from selected districts in west Arsi zone of Oromia regional state namely; Kofale and Shashemene. Out of these animals, 165 were from Shashemene, 193 were from Kofale. Breed, age, parity, hygiene of udder, tick infestation of udder, lactation stage, floor, husbandry system, sequence of milking of cows were explanatory variables used to associate with prevalence rate.

2.3. Study Design: A cross-sectional study was conducted from October 2014 to April 2015 to estimate the prevalence of mastitis, isolate and identify major bacterial pathogens and to assess the association of some potential risk factors with

occurrence of mastitis in lactating cows in Shashemene and Kofale, west Arsi zone of Oromia regional state.

2.4. Sampling and Sample Size Determination:

Purposive sampling technique was employed to selected two districts from 12 districts of the zone based on dairy cow population. The peasant association and lactating cows were also selected purposefully based on their accessible for transportation. The sample size was determined based on the formula given by Thrusfield (2007) considering 5% absolute precision, 95% confidence interval and from previous studies in the study area (Abera *et al.*, 2012), with an expected prevalence of 37%. Therefore, the total sample size was 358 based on the given formula below.

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

Where, n = required sample size

P_{exp} = expected prevalence

d = desired absolute precision

2.5. Study Methodology

Based on the clinical inspection and CMT result cases were categorized as positive or negative and positive cases were again categorized as subclinical and clinical mastitis Quinn *et al.* (1999). Lactation stage, parity, sequence of milking, type of floor, husbandry system and age of study animals were obtained by interviewing the owner of the herd. Lactation stage was categorized as early (1 up to 3 month), mid (4 up to 6 month) and late (>7 months); age as young adult (3 up to 5 year), adult (6 up to 9 year) and old (9 year and above); parity as few (1 up to 3 calves), moderate (4 up to 6 calves), and many (above 6 calves).

2.5.1. Data collection: A questionnaires were used to collect data on the possible risk factors to the mastitis prevalence such as; breed, presence of lesion on skin of udder, and /or teats; parity number and hygiene of milking process as good and poor (that is based on udder washing before milking, drying of udder with cloth before milking and washing of milkers' hand before milking or not).

2.5.2. Physical examination of udder and milk: The udder of the study cows was examined visually and by Palpation for presence of clinical mastitis. During examination attention was paid to cardinal signs of inflammation, symmetry, size and consistency of udder quarters (Radostits *et al.*, 2007).

2.5.3. Californian mastitis test: The California mastitis test (CMT) was conducted to diagnose the presence of subclinical mastitis and it was carried out according to the procedures given by Quinn *et al.*

(1999). The udder of the cow to be tested was cleaned with warm water and antiseptics and was dried with clean towel. Then the first few drops were discarded from each quarter. Following that squirt of milk from each quarter of udder, milk samples were poured in to four shallow cups in the CMT paddle and equal amount of CMT reagent was added to each cup and gentle circular motion was applied to the mixture on the horizontal plane. Based on the thickness of the gel formed by CMT reagent and milk mixture, test results were scored as 0 (negative), 1 (weak positive), 2 (distinct positive) and 3 (strong positive). Milk samples with test result of CMT 1 to 3, were classified as evidence of subclinical mastitis (Radostits *et al.*, 2007; Quinn *et al.*, 1999).

2.5.4. Sample collection and bacteriological examination:

Milk samples were collected after the skin of the udder was washed with tap water and dried with clean towel and the teat were swabbed with cotton soaked in 70% ethyl alcohol. The samples were collected in sterile universal bottles held nearly horizontal, after discarding the first few squirts of milk (Quinn *et al.*, 1999). All sample bottles were labeled for cow identification and for the respective quarters. Milk samples were immediately transported to the microbiology laboratory of the National Animal Health and Investigation Center (NAHDIC) in ice packed cool box for bacteriological analysis. All milk samples were subjected to bacteriological examination according to Quinn *et al.* (1999) commencing on the date of collection.

2.5.5. Culture and isolation of bacteria: In the laboratory a loop full of the milk sample was streaked on blood agar base enriched with 7% sterile sheep blood and MacConkey agar and incubation was made at 24 to 48 hours. Then plates were examined for growth, colony morphology and hemolytic characteristics on blood agar. Subcultures were done to obtain pure isolates for further identification. Culture positive plates were identified according to gram stain reaction, colony morphology and catalase test. Identification of bacterial species was done according to Quinn *et al.* (1999) using standard methods. Oxidase test was done to distinguish *Enterobacteriaceae* from other gram negative bacteria. *Enterobacteriaceae* were subjected IMVIC test for species level identification.

2.6. Data Analysis and Management: Data collected during the study period were entered into Microsoft Excel 2007 spread. STATA 11 statistical software was used for the analysis. Logistic regression was used to see the association between different risk factors like: breed, age hygiene of milking, parity, lactation stage p <0.05 were considered as significant.

3. Results

3.1. Prevalence of Bovine Mastitis: From the total of 358 lactating cows examined, 38% (136/358) were positive for mastitis. Of these, 7.3% (26/358) and 30.7% (110/358) were found to be positive for clinical mastitis and subclinical mastitis based on the clinical diagnosis and CMT, respectively. The study also

considered mastitis at quarter level. An overall prevalence of quarter level was 34.5% (490/1422); from this the clinical form was 6.6% (94/1422) and the subclinical was 27.9% (396/1422). Out of the 1432 quarters examined, 10 had blind teats (Table 1).

Table 1: Prevalence of clinical and sub clinical mastitis at cow and quarter levels

Cow level (n=358)			Quarter level (n=1422)	
Types of mastitis	No. of positive	Prevalence (%)	No. of positive	Prevalence (%)
Clinical	26	7.3	94	6.6
Subclinical	110	30.7	396	27.9
Total	136	38	490	34.5

Total animal examined in Shashemene were 165, out of these 35.8% (59/165) animals were positive for mastitis, from these, 8.5% (14/165) clinical and 27.3% (45/165) subclinical mastitis while 193 animals were

examined in Kofale, out of these 39.9% (77/193) animals were positive for mastitis, from these 6.2% (12/193) clinical and 33.7% (65/193) subclinical mastitis (Table 2).

Table 2: Summary of prevalence of clinical and subclinical mastitis at cow level in study area

Districts	No. of cow examined	Total No. of cows affected	Over all Prevalence (%)	Form of mastitis			
				Clinical		Subclinical	
				No. of positive	Prevalence (%)	No. of positive	Prevalence (%)
Shashemene	165	59	35.8	14	8.5	45	27.3
Kofale	193	77	39.9	12	6.2	65	33.7
Total	358	136	38	26	7.3	110	30.7

3.2. Result of bacterial isolation: Analysis of bacteriological examination of milk samples was made to identify the main etiological agents involved in the disease. The organisms were identified on the basis of their cultural, staining characteristics and biochemical reactions. Milk sample of 101 quarters, which were positive for CMT, cultured for microbiological examination in the study period, about 7 bacterial species and 83(82.2%) bacterial isolates. The bacterial

isolation rate and their prevalence are shown on (Table 3). The predominant isolated bacteria were *Staphylococcus aureus* with isolation rate of 44.6% followed by *Streptococcus agalactiae* with isolation rate of 18.1%, CNS (coagulase negatives *Staphylococcus* species) (that was the third predominant isolated with isolation rate of 16.9%). *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were the least isolate which accounts for 3.6% each.

Table 3: Bacterial species isolated from the study area

Bacterial species	Total number of isolates	Prevalence (%)
<i>Staphylococcus aureus</i>	37	44.6
CNS	14	16.9
<i>Streptococcus agalactiae</i>	15	18.1
Other <i>Streptococcus</i> species	7	8.4
<i>E.coli</i>	4	4.8
<i>Pseudomonas aeruginosa</i>	3	3.6
<i>Klebsiella pneumonia</i>	3	3.6
Total	83	100

CNS = Coagulase Negative *Staphylococcus*

3.3. Risk factors: Among the different potential risk factors considered for a univariate logistic regression, all the risk factors like: breed, age, parity, tick infestation of udder, sequence of milking, floor, husbandry system, lactation stage and hygiene of udder

were found statistically significant ($P < 0.05$). However, all became insignificant, except milking hygiene and stage of lactation when tested with multivariate logistic regression (Table 4). Both stage of lactation and milking hygiene were found to be statistically

significant ($P < 0.05$) associated with the occurrence of mastitis. Mastitis prevalence was found to be higher in early lactation and lower in mid lactation stages (Table 4). Statistical analysis showed the existence of significant difference ($P < 0.05$) between the occurrence of mastitis and lactation stage. The occurrence of mastitis was higher in poor milking hygiene and lower

at good milking hygiene. Furthermore, Animals over 8 years old were more frequently affected with the disease and those younger than 5 years were rarely affected. Also the highest prevalence of mastitis was observed in animals with parity of more than 6, followed by 3-6 and 1-2 parity (as indicated Table 4).

Table 4: Analysis result of risk factors for the occurrence of bovine mastitis in the study area

Risk factors	Category	No. of cows examined	No. of cows affected	Prevalence (%)	Crude Odds ratio (95 % CI)	Adjusted Odds ratio (95% CI)	P-value
Breed	Local	169	41	24.3	-	-	-
	Cross	189	95	50.3	0.39(0.1583-0.9442)	0.75(0.2475-2.2640)	0.608
Age	3-5years	176	8	4.5	-	-	-
	5-7years	88	43	48.9	0.20(0.0506-0.7960)	1.64(0.2503-10.7028)	0.607
	>8 years	94	85	90.4	0.09(0.0239-0.2986)	0.98(0.1842-5.2174)	0.982
Parity	1-3 calves	122	15	12.3	-	-	-
	4-6 calves	124	36	29	0.16(0.0193-1.3706)	0.32(0.3091-3.2693)	0.335
	>6 calves	112	85	75.9	0.04(0.0053-0.3077)	0.18(0.0193-1.7363)	0.139
Lactation stage	1-3 month	174	65	37.4	-	-	-
	4-6 month	144	45	31.3	0.82(0.3159-2.1188)	1.23(0.3271-4.626)	0.759
	>7 month	40	26	65	0.22(0.0783-0.6080)	0.08(0.0074-0.8448)	0.036
Floor	Good concrete	166	12	7.2	-	-	-
	Muddy soily	148	100	67.6	0.08(0.0189-0.3619)	0.54(0.9674-2.9667)	0.475
	Bad concrete	44	24	54.5	.09(0.0178-0.5086)	0.39(0.0542-2.7958)	0.348
Husbandry system	Extensive	146	35	24.0	-	-	-
	Intensive	156	62	39.7	0.31(0.1107-0.8700)	0.46(0.0956-2.261)	0.343
	Semi-intensive	56	39	69.6	0.36(0.1005-1.3013)	12.45(0.8666-178.9095)	0.064
Sequence of Milking of cows	1 st Health,last mastitis	169	6	3.6	-	-	-
	Arbitrary	189	140	74.1	0.13(0.0384-0.4428)	0.56(0.1095-2.8609)	0.486
Milking hygiene	Neither washed nor Disinfected	124	105	84.7	-	-	-
	only washed	60	20	33.3	2.85(0.9335-8.7279)	1.47(0.3861-5.6199)	0.571
	Washed and dried with towel	174	11	6.3	35.27(4.6749-266.1197)	10.38(1.1406-94.5100)	0.038
Tick infestation of udder	Absent	189	13	6.9	-	-	-
	Present	169	123	72.8	0.15(0.0487-0.4285)	0.47(0.1073-2.0375)	0.311

4. Discussion

The study was carried out to determine the prevalence of bovine mastitis and to identify the major bacterial causes of mastitis in Shashemene and Kofale

districts on 358 animals and the overall prevalence was 38%. This result agree with the findings of Abera *et al.* (2012), Nessru(1999), Workineh *et al.* (2002), Fekadu(1995),who reported 37.1% in Shashemene,

37.2 in urban and peri-urban dairy farms at Addis Ababa, 38.2% in Adami Tulu, 38.65% in Chaffa valley respectively. However, it is relatively lower than the reports of Mekibib *et al.* (2010), Lakew *et al.* (2009), Sori *et al.* (2005) and Mungube *et al.* (2004) who recorded 71.1% from Holeta, 64.6% from Assela, 52.8% from Sebeta and 46.6% from central highlands of Ethiopia, respectively. Mastitis is a complex disease and the difference in results might be due to differences in management system of the farm, the breeds of cattle considered, technical know-how of the investigators and the geographical locations of the studies.

The present study revealed with prevalence of subclinical mastitis (30.7%) and clinical mastitis (7.3%) which is comparable with the findings of Nessru (1999) and Bishi (1998), who reported 32.2% subclinical and 5% clinical in urban and peri-urban dairy farms at Addis Ababa, 30.2% subclinical and 5.5% clinical in urban and peri-urban in and around Addis Ababa, respectively. Prevalence of subclinical mastitis is higher than that of clinical mastitis in the present study which is in the agreement with several earlier reports from different parts of Ethiopia (Abera *et al.*, 2010; Mekibib *et al.*, 2010; Lakew *et al.*, 2009; Almaw *et al.*, 2008; Getahun *et al.*, 2008; Biffa *et al.*, 2005; Mungube *et al.*, 2004; Kerro and Tareke, 2003; Workineh *et al.*, 2002) and elsewhere in Africa (Kivaria *et al.*, 2004). Since, environmental factors play significant role, the prevalence of subclinical mastitis varies in dairy animals (Radostits *et al.*, 2007). Subclinical mastitis has been reported to be higher than clinical mastitis owing to the defense mechanism of the udder, which reduces the severity of the disease (Erskine, 2001). In most reports including the present study, clinical mastitis is far lower than subclinical mastitis (Sori *et al.*, 2005; Biffa *et al.*, 2005; Almaw *et al.*, 2008; Lakew *et al.*, 2009; Haftu *et al.*, 2012). This could be attributed to little attention given to subclinical mastitis, as the infected animal shows no obvious symptoms and secretes apparently normal milk and farmers, especially small holders, are not well informed about invisible loss from subclinical mastitis. In Ethiopia, the subclinical forms of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases (Almaw *et al.*, 2008).

Quarter prevalence of mastitis 34.5% found in this study was comparable with the finding of Nessru *et al.* (1997) and Bachaya *et al.* (2011) in Pakistan, who reported the 37%, and 35.25%, but higher than the report made by Biffa *et al.* (2005) who reported quarter prevalence of 28.2%.

From the 101 CMT positive quarter milk samples, 83(82.2%) were bacteriologically positive up on culturing, while 18 (17.8%) were bacteriologically

negative, which is in line with the results of Aregaw (1992), who reported 18% bacteriologically negative samples, however; higher than the reports of Sori *et al.* (2005) who reported a prevalence of 9.82%. The failure to isolate bacteria from the CMT positive milk samples could be partly associated with spontaneous elimination of infection, low concentration of pathogen in milk, intermittent shedding of pathogen, intracellular location of pathogens and presence of inhibitory substance in milk (Radostits *et al.*, 2000). It might also be due to some cases of delayed healing of infection from which organisms may have disappeared or reduced, while infiltration of leukocytes continued until complete healing (Sori *et al.*, 2005).

In this study, most of the bacterial pathogens isolated from milk samples were *Staphylococcus* species, *S. aureus* (44.6%) comparable with the finding of Abera *et al.* (2010) who report of (42.1%). The relative high prevalence of *S. aureus* in the current study shows the absence of dry cow therapy and low culling rate of chronically infected animals practice in the study area. The bacteria usually establish chronic, subclinical infections and are shed in the milk, which serves as a source of infection for other healthy cows during the milking process. *Streptococcus* species were also found prevalent with 26.5% share of the total isolates: *Streptococcus agalactiae* 18.1% and other *Streptococcus* species 8.4%. This finding coincides with that of Hawari and Al-dabbas (2008), and Zerihun (1996), who reported 26.2% in Jordan, 27% in Stela Dairy Farm, Ethiopia, respectively. However, the finding is higher than the reports of Bitew *et al.* (2010) at Bahir Dar and its environ (13.9%) and Sori *et al.* (2005) in and around Sebeta (3.73%) and lower than the report of Atyabi *et al.* (2006) at farms around Tehran (33.54%). The predominance of these two bacterial species (*Staphylococcus* and *Streptococcus*) is due to frequent colonization of teats as they are commensals of the skin. Then they can easily get access to the teat canal during milking or suckling and can be transmitted from quarter to quarter and from cows to cows during milking practices. Their ability to exist intracellularly and localize within micro-abscessation in the udder and hence, resistant to antibiotic treatment (MacDonald, 1997) could also be important factor contributing to the predominance of these organisms.

E. coli were the predominant bacteria among the coliforms with an isolation rate of 4.82% in this study which is in consent with the observations of Mekuria (1986) and Biffa (1994) who reported 4.60%, 3.64% and 3.14%, respectively from different parts of Ethiopia. In this study, *Klebsiella* species accounted for 3.6% among coliforms.

There is also other coagulase negative *Staphylococcus* which contributes about 16.9% of the

isolates which is in line with the report of Sori *et al.* (2005) in and around Sebeta (14.93%).

The occurrence of bovine mastitis and lactation stage was significantly ($p < 0.05$) associated. That is, higher infection in cows in early lactation stage followed by late and medium lactation stages, that concurs with previous reports (Biffa *et al.*, 2005). The early lactation stage infection might be due to the carryover of infection from dry period. In cows most new infections occur during the early part of the dry period and in the first two months of lactation (Radostits *et al.*, 2007). Absence of dry cow therapy regime could possibly be the major factor contributing to high prevalence at early lactation and early infection associated with delayed diapedesis of neutrophils in to the mammary gland (Schalm *et al.*, 1971).

Prevalence of mastitis was significantly ($p < 0.05$) associated with milking hygienic practice. Cows at farms with poor milking hygiene standard are severely affected than those with good milking hygiene practices (Lakew *et al.*, 2009; Sori *et al.*, 2005). This might be due to absence of udder washing, milking of cows with common milkers' and using of common udder cloths, which could be vectors of spread especially for contagious mastitis.

5. Conclusion and Recommendations

The present study showed that an overall prevalence of 38% bovine mastitis was recorded in the study area. Milk sample of 101 quarters, which were positive for CMT were cultured for microbiological examination in the study period, about 7 bacterial species and 83(82.2%) bacterial isolates were found. The predominant isolated bacteria were *Staphylococcus aureus* with isolation rate of 44.6% followed by *Streptococcus agalactiae* with isolation rate of 18.1%. Among the different potential risk factors considered for univariate logistic regression all the risk factors like: breed, age, parity, tick infestation of udder, sequence of milking, floor, husbandry system, lactation stage and hygiene of udder were found statistically significant ($P < 0.05$). However, all became insignificant, except milking hygiene and stage of lactation when tested with multivariate logistic regression. Mastitis is still the most important disease of dairy animals in the study area.

In line with above facts the following recommendations are forwarded:

- Awareness creation should be given to the dairy herds on the impacts of bovine mastitis
- All quarters of the udder of each cow should be periodically checked for the timely treatment and prevention.

➤ To confirm the presence of sub clinical mastitis in the herd bacterial culture, SCC and CMT should be used.

➤ As there is no sufficient information about bovine mastitis in the study area, detailed and organized studies should be under taken to come up with relevant and appropriate control and preventive measurement.

Acknowledgements

The authors would like to thank Wollega University for the financial support towards this research. Grateful acknowledgements are also extended to National Animal Health and Investigation Center and Admi Tulu Agricultural Research Center for their kind cooperation in the laboratory work, providing vehicles and other necessary materials.

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References

1. Central Statistical Authority (CSA). Report on livestock and livestock characteristics; Volume II, Ethiopia, Addis Ababa, 2014.
2. Biffa D, Debela E, Beyene F. Prevalence and risk factors of mastitis in lactating dairy cows in southern Ethiopia. *International Journal of Application Research in Veterinary Medicine*. 2005; 3:189-198.
3. Mohamed AM, Ehui S, Assefa Y. Dairy development in Ethiopia, International food policy research institute, 2033K street, Washington, DC 2006 USA. Discussion, 2004.
4. Felleke G, Geda G. The Ethiopia Dairy development policy a draft policy document, Addis Ababa, Ethiopia: Ministry of Agricultural Organization, 2001.
5. Vallezarate A. Per-urban live stock production in Ethiopia: A case study on per-urban dairy production around Addis Ababa. Abgeschlossene dissertation, University of Hohenheim, 2000.
6. FAO. Livestock Sector Brief. Livestock information, Sector Analysis and Policy Branch, 2003; 1-15.
7. Vaarst M, Envoldsen C. Patterns of clinical mastitis in Danish organic dairy herd. *Journal of Dairy Science*. 1997; 64:23.
8. Nibret M. Bovine Mastitis and Associated Risk Factors in Small Holder Lactating Dairy Farms in Hawassa, Southern Ethiopia, 2009.
9. Radostits OM, Gay CC, Hinchcliff KW, Constable PD. Mastitis in Veterinary medicine:

- Text book of the disease of cattle, horse, sheep, pigs and goats 10th Ed. Elsevier London, 2007; 674-762.
10. Robertson JR, Fox LK, Hancock DD, Gay JM, Besser TE. Ecology of *Staphylococcus aureus* isolated from various site on dairy farms. *Journal of Dairy Science*, 1985; 77:3354.
 11. Stephen R, Annemuller C, Hassan AA, Laemmler CH. Characteristic of entero toxigenic *Staphylococcus aureus* strain isolated from bovine mastitis in north-east Suizerland. *Veterinary Microbiology*. 2001; 78:373-382.
 12. Fekadu K, Survey on prevalence of bovine mastitis and the predominant causative agents in Chaffa Valley. 1995; 101-111.
 13. Mekonnen H, Workineh S, Bayleyegne M, Tadele K. Antimicrobial susceptibility profiles of mastitis isolated from cows in three Ethiopian dairies. *Revue .veterinary medicine*. 2005; 7: 391- 394.
 14. Kassa T, Wirtu G, Tegegne A. Survey of mastitis in dairy herds in the Ethiopian central highlands. *Ethiopia Journal Science*. 1999; 22: 291-301.
 15. Lemma M, Kassa T, Tegegne A. Clinically manifested major health problem of cross-breed dairy herds in urban and pre-urban production system in the central highlands of Ethiopia. *Tropical Animal Health Production*. 2001; 33: 85-89.
 16. Mungube E O. Management and economics of dairy cow mastitis in the urban and pre-urban areas of Addis Ababa University, Debra Zeit, Ethiopia. MSc thesis, 2001.
 17. Kerro O, Tareke F. Bovine mastitis in selected areas of southern Ethiopia. *Tropical Animal Health Production*, 2003; 35: 197-205.
 18. Workineh S, Bayleyegn M, Mekonnen H, Potgiete, LND. Prevalence and etiology of mastitis in cows from two major Ethiopian dairies. *Kluwer Academic Publishers*, 2002; Netherland.
 19. Tamirat TA. Comparison of clinical trials of bovine mastitis with the use of honey, MSc thesis, Addis Ababa University, Ethiopia, 2007; 14-30.
 20. Almaw G, Molla W, Melaku A. Prevalence of bovine subclinical mastitis in Gondar town and surrounding areas, Ethiopia. *Livestock Research for Rural Development*, 2009.
 21. Bitew M, Tafere A, Tolosa T. Study on Bovine Mastitis in Dairy Farms of Bahir Dar and its Environs. *Journal of Animal Veterinary Advanced*, 2010; 9:2912-2917.
 22. Abera M, Elias B, Aragaw K, Denberga Y, Amenu K., Sheferaw D. Major causes of mastitis and associated risk factors in smallholder dairy cows in Shashemene, southern Ethiopia. *African Journal of Agricultural Research*, 2012; 7:3513-3518.
 23. SDARDO. Shashemene Districts Agriculture and Rural Development Office, the Annual Report. Shashemene, Ethiopia, 2010.
 24. KDARDO. Kofale Districts Agriculture and Rural Development Office, the Annual Report. Kofale, Ethiopia, 2010.
 25. Central Statistics Agency (CSA). Federal Democratic Republic of Ethiopia population census commission: Summary and statistical report of the 2007 population and housing census, 2008.
 26. Thrufield M. *Veterinary Epidemiology*, 3rd edition, London: Blackwell Science, a Blackwell publishing company, 2007; 214-265.
 27. Quinn PJ, Carter ME, Markey BK, Carter GR. *Clinical veterinary microbiology*. Mosby International Limited, London, 1999; 327-344.
 28. Nessru H. A cross-sectional and longitudinal study of bovine mastitis in urban and pre-urban dairy system in Addis Ababa region. Free University of Berlin and Addis Ababa University, Ethiopia, MSc, thesis, 1999.
 29. Mekibib B, Furgasa M, Abunna F, Megersa B, Regassa A. Bovine Mastitis, Prevalence, Risk Factors and Major Pathogens in Dairy Farms of Holeta town. Central Ethiopia. *Veterinary World*, 2010; 9: 397 - 403.
 30. Lakew M, Tolosa T, and Tigre W. Prevalence and major bacterial causes of bovine mastitis in Asella, South Eastern Ethiopia. *Tropical Animal Health Production*, 2009; 41: 1525-1530.
 31. Sori H, Zerihun A, Abdicho S. Dairy Cattle Mastitis in Sebeta, Ethiopia. *International Journal of Veterinary Medicine*, 2005; 3:332-338.
 32. Mungube ED, Tenhagen BA, Kassa T, Regessa F, Kyule MN, Greiner M, Baumann MPO. Risk factors for dairy cows in the central highland of Ethiopia. *Tropical Animal Health and Production*, 2004; 36: 463-472.
 33. Bishi AB. Cross-section and longitudinal prospective study of bovine clinical and subclinical mastitis in peri-urban dairy production system in Addis Ababa region FVM, Addis Ababa University; Debre Zeit., Msc thesis, 1998.
 34. Abera M, Demie B, Aragaw K, Regassa F, Regassa A. Isolation and identification of *Staphylococcus aureus* from bovine mastitic milk and their drug resistance patterns in Adama town, Ethiopia. *Journal of Veterinary Medicine and Animal Health*, 2010; 2: 29-34.

35. Almaw G, Zerihun A, Asfaw Y. Bovine mastitis and its association with selected risk factors in smallholder dairy farms in and around Bahir Dar, Ethiopia. *Tropical Animal Health Production*, 2008; 40: 427-432.
36. Getahun K, Belihu K, Bekana M, Lobago F. Bovine mastitis and antibiotics resistance pattern in Selalle smallholder dairy farms, Central Ethiopia. *Tropical Animal Health Production*, 2008; 40: 261-268.
37. Kivaria FM, Noordhuizen JP, Kapaga AM. Risk indicators associated with subclinical mastitis in smallholder dairy cows in Tanzania. *Tropical Animal Health Production*, 2004; 36: 581-592.
38. Erskine RJ. Food Animal Production Medicine 3rd ed. W. B. Saunders Company, Philadelphia. 2001; 397-435.
39. Haftu R, Taddele H, Gugsa G, Kelayou S. Prevalence, bacterial causes, and antimicrobial susceptibility profile of mastitis isolates from cows in large-scale dairy farms of Northern Ethiopia. *Tropical Animal Health Production*, 2012; 44:1765-1771.
40. Nessru H, Teshome Y, Getachew T. Prevalence of mastitis in cross-bred and zebu cattle. *Ethiopia Journal of Agricultural Science*, 1997; 16: 53.
41. Bachaya HA, Raza MA, Murtaza S, Akbar IU R. Subclinical bovine mastitis in Muzaffargarh district of Punjab (Pakistan). *The Journal Animal and Plant Science*, 2011; 21:16-19.
42. Aregaw M. Incidence of mastitis and its influence on milk yield and composition in Debre zeit. DVM Thesis, Addis Ababa University, FVM, Ethiopia, 1992.
43. Radostits OM, Gay GC, Blood DC, Hinchillif KW. *Veterinary Medicine*, 9th Edition, Harcourt Limited, London, 2000; 603-700.
44. Hawari D, Al-dabbas F. Prevalence and distribution of mastitis pathogens and their resistance against anti-microbial agents in dairy cows in Jordan. *American Journal of Animal and Veterinary Science*, 2008; 3:36-39.
45. Zerihun T. A Study on Bovine Subclinical Mastitis at Stela Dairy Farm, Ethiopia DVM, thesis, Addis Ababa University, Faculty of Veterinary medicine, Ethiopia, 1996; 45- 52.
46. Atyabi N, Vodjgani M, Gharagozloo F, Bahonar A. Prevalence of bacterial mastitis in cattle from the farms around Tehran. *Iranian Journal Veterinary Medicine*, 2006; 7: 76-79.
47. MacDonald US. Streptococcal and Staphylococcal mastitis. *Journal of American Veterinary Medicine Association*, 1997; 170:1157.
48. Mekuria M. Prevalence and etiology of bovine mastitis in Bahir Dar, Faculty of Veterinary Medicine, Addis Ababa University, DebreZeit, DVM Thesis, 1986.
49. Biffa D. A study on the prevalence bovine mastitis in indigenous zebu cattle and Jersey breeds in Wallaita Sodo. Characterization and *invitro* drug sensitivity of isolate. DVM thesis, Addis Ababa University, Ethiopia, 1994.
50. Schalm DW, Carroll EJ, Jain NC. *Bovine Mastitis*. Lea and Febiger. Philadelphia, 1971; 182-282.

7/9/2015