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Prevalence, Risk Factors and Major Bacterial Causes of Bovine Mastitis in Smallholder Dairy Farms in and around Sinana District, Bale Zone, South Eastern Ethiopia

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Keywords: *bacterial isolates, bovine mastitis, lactating cow, prevalence, risk factors, sinana.*

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Prevalence, Risk Factors and Major Bacterial Causes of Bovine Mastitis in Smallholder Dairy Farms in and around Sinana District, Bale Zone, South Eastern Ethiopia

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Abstract- A cross-sectional study was conducted from November 2013 to May 2014 on lactating dairy cows to determine the overall prevalence of bovine mastitis, identify associated risk factors and isolate the predominant bacterial agents involved in causing mastitis in and around Sinana district. A total of 384 lactating cows were examined for mastitis using clinical examination and California Mastitis Test (CMT). Bacteriological isolation techniques were also undertaken to recover the causative bacterial pathogens. Prevalence of mastitis at cow level was 36.72%, out of which 4.95% and 31.77% were clinical and subclinical cases, respectively. The quarter level prevalence was 26.43%; from this, the clinical and subclinical forms were 2.28% and 24.15%, respectively. Out of total examined teats, 1.30% was blind. About 356 bacterial isolates identified from mastitic milk samples. The isolates based on their relative frequency of occurrence were: *Staphylococcus aureus* (33.99%), *Streptococcus agalactiae* (24.44%), *Staphylococcus epidermidis* (10.96%), Coagulase-Negative Staphylococci (CNS) (7.58%), *Escherichia coli* (6.46%), *Streptococcus dysgalactiae* (6.18%), *Corynebacterium bovis* (5.34%), *Klebsiella pneumonia* (2.81%) and *Bacillus cereus* (2.23%). Risk factors analysis revealed that prevalence of mastitis was significantly differed with the age (P< 0.01), parity (P< 0.05), breed (p<0.001), stage of lactation (p<0.001), mastitis record (p<0.01), dry cow therapy (p<0.05), udder hygiene (p<0.01), drainage system (p<0.05), floor type (p<0.05) and grazing system (P<0.05). Thus, prevalence was relatively higher in adult cows (OR = 1.784; 95% CI = 0.999, 3.189), multiparous cows (OR = 1.320; 95% CI = 0.552, 3.155), cross breed cows (OR = 5.820, 95%CI = 3.248, 10.430), early stage lactation (OR=3.021, 95%CI=1.617, 5.647), late stage lactation (OR = 3.280, 95%CI = 1.931, 5.572), cows with history of mastitis (OR = 2.452, 95%CI = 1.282, 4.688), cows untreated during drying off (OR=1.445, 95%CI=0.467, 4.473), cows with unwashed udder (OR = 13.386, 95% CI = 1.300, 137.845) and cows under zero grazing (OR=1.892, 95%CI=1.022, 3.501) than those corresponding animals. Generally, the study showed that mastitis is an important problem and a serious threat for the dairy industry in the study area. Therefore, appropriate control measures targeting the specific causative agents should be in place to reduce the impact of the disease. The farmers should have to implement sound management practices that improve udder and teat heath problems.

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Keywords: bacterial isolates, bovine mastitis, lactating cow, prevalence, risk factors, sinana.

I. Introduction

Bovine mastitis is the inflammation of the mammary gland often due to microorganisms that attack the udder, proliferate and release toxins that are injurious to the udder and teat tissues (Schroeder, 2012). It has been a disease of cattle for probably as long as humankind has milked cows (Erskine *et al*., 2002). Mastitis is among the most significant diseases in dairy animals with worldwide distribution (Zhao and Lacasse, 2007). It is manifested by un array of physical and chemical alterations in the milk and pathological lesions in the glandular tissue (Radostits *et al*., 2007). It is a global problem responsible for massive financial losses to dairy industries and economies at large due to poor milk quality, reduced milk yield and increased expenditure on treatment and sometimes death due to the disease itself or through culling of affected cows (Schroeder, 2012).

Numerous microorganisms have been described as causative agents of bovine mastitis (Watts, 1988; Bradley, 2002). According to their epidemiology, mastitis pathogens can be divided into contagious and environmental. The primary reservoir of contagious pathogens is an infected udder whereas a contaminated environment is the primary reservoir of pathogens causing environmental mastitis. *Staphylococcus aureus, Streptococcus agalactiae*, and Mycoplasma species are considered as typical contagious pathogens. Typical environmental pathogens are streptococci (streptococci other than *Streptococcus agalactiae* such as *Streptococcus uberis*; enterococci), Enterobacteriaceae and coagulase-negative staphylococci (CNS). *Streptococcus dysgalactiae* has been most commonly considered as a contagious pathogen, but it can also act as an environmental pathogen (Gruet *et al*., 2001; Bradley 2002; Barkema *et al*., 2009). Likewise, the contagious infection has also been recorded in certain coagulase-negative staphylococci (CNS) (Gillespie *et al*., 2009). Pathogens such as Pseudomonas species, Pasteurellaceae, some pyogenic and anaerobic

bacteria, yeasts and algae number among those which occur occasionally. In current times, there is obvious confirmation for rising occurrence of environmental mastitis while the incidence of contagious mastitis has decreased (Bradley 2002; Rysanek *et al*., 2007).

Intra-mammary infections (IMI) can result in mastitis which is either sub-clinical or clinical. Clinical mastitis is type of mammary tissue infection that can be directly seen, with signs such as alterations in milk composition and appearance; reduction in milk production; affected udder/teats become red, hard, hot and swollen. In addition, it is manifested by symptoms like increased in body temperature, rapid pulse, loss of appetite, depression and sometimes death. Sub-clinical mastitis is generally defined as the absence of visible symptoms but characterized by cell count (SCC) of greater than 2.5 x105 cells/ml (Schukken *et al.,* 2003) or the presence of a known pathogen in the secreted milk as detected by culture. Subclinical form commonly found in most herds (Gruet *et al.,* 2001; Awale *et al*., 2012). Clinical mastitis is mainly caused by pathogens such as *Streptococcus uberis*, *Escherichia coli*, Klebsiella species, *Pseudomonas aeruginosa* and pyogenic bacteria. On the other hand, *Streptococcus agalactiae*, Coagulase-negative Staphylococci (CNS) and Enterococcus species are associated with subclinical mastitis (Bradley 2002; Barkema *et al*., 2009; Awale *et al*., 2012). However, *Staphylococcus aureus* has been considered as the cause of both clinical (Gruet *et al*., 2001) and subclinical mastitis (Awale *et al*., 2012). In contrary to the clinical form of the disease, subclinical mastitis is difficult to recognize, and for this reason, it may result in heavy losses in milk yield. In addition, subclinically affected cows might represent a source of particular pathogens that can be spread via automatic milking systems (Barkema *et al*., 2009; Hovinen and Pyorala 2011).

The incidence of mastitis is significantly influenced by environment and management related factors (Steeneveld *et al*., 2008; Ali *et al*., 2014). The occurrence of mastitis depends on three components which include exposure to microbes, cow defense mechanism, environmental and management factors (Suriyathaporn *et al*., 2000). The early months of lactation is the most sensitive period for mastitis risk in the cow even in the well-managed herds (Andrew *et al*., 2004). Numerous risk factors with bovine mastitis are associated microflora of the udder, udder shape and condition, teat injuries, teat length, increasing teat canal diameter, udder depth, teat morphology (Tiwari *et al*., 2013; Ali *et al*., 2014). Majority of diagnosed mastitis cases are the result of bacterial infections. A major survey of New York and Pennsylvania dairy herds found that almost 50% of all cows were experiencing some form of mastitis caused by a culturable microorganism; less than 1 % of these were due to a non-bacterial pathogen (Wilson *et al.,* 1997). These pathogens invade the mammary glands, develop and multiply, producing some toxic substances that result in inflammation, reduced milk production and altered milk quality leading to a clinical condition known as mastitis (Oliver and Muranda, 2012; Rall *et al*., 2013).

The existing literatures revealed that udder and teat disease is one of the most regularly encountered diseases of dairy cattle. Investigation conducted by Lemma *et al*. (2001) showed, of the main diseases of cross breed cows in Addis Ababa milk shed, clinical mastitis was the second most frequent next to reproductive disease. Mastitis, as a disease, has received little attention in Ethiopia, especially the sub clinical form (Mekonnen *et al*., 2005; Hundera *et al*., 2005) which occurs at a much higher rate than clinical mastitis, yet it is the nastiest in terms of reduced productivity (Quinn *et al*., 2002). Owing to the serious financial insinuation involved and the predictable existence of latent infection, mastitis is the vital factor that limits dairy industry. There are various reports indicating a high prevalence of bovine mastitis in dairy farms in different parts of Ethiopia (Mekibib *et al*., 2010; Bedada and Hiko, 2011; Fentaye *et al*., 2014; Tilahun & Aylate, 2015; Teklemariam *et al*., 2016).

Although various investigations have been conducted on bovine mastitis in Ethiopia so far, the problem is still challengeable for the bovine mastitis researchers and particularly for field veterinarians to treat and control it. Now there is a need to imply the strategic control measures for this deadly disease of dairy animals to prevent heavy economic losses of farmers. We need distribution and changing trend of etiological agents, prevalence and potential risk factors of mastitis in the study area to apply strategic plan for control of mastitis. Moreover, there is no published data on status, magnitude, and distribution of mastitis in Bale Zone in general and in and around Sinana district in particular. Hence, the aim of this investigation is to establish the distribution of etiological agents, prevalence and potential risk factors of bovine mastitis from the study area.

II. Materials and Methods

a) *Description of the Study Area*

The study was conducted in and around Sinana district of Bale zone, Oromia Regional State, South Eastern Ethiopia. It is located at 430 km south-east of Addis Ababa. The area is located at 7O7' N and 40O10' E and 2400 meters above sea level. The mean average rainfall of the district is 353 mm. Moreover, an average annual maximum temperature is 21.2oC, and the minimum temperature is 9.4oC. The agricultural production system of the study area is mixed farming. There are about 251,489 heads of cattle, of which 59,561 are dairy cows, 47,121 Sheep, 10,300 goats, 9,163 horses, 14,015donkey, 2,800 mules, 59,655

poultry and 13,690 beehives in Sinana woreda (Sinana Woreda Agricultural and Rural Development Office, 2013). Dairy farming using local and improved (cross) breeds is a common practice in Sinana district where dairy production plays a crucial role in the livelihood of the farming community. The management system of dairy cows is mainly extensive in rural areas and intensive in town. Traditional housing, feeding and milking procedures are mostly practiced.

b) Study Population and Animals

The study populations were all lactating cows from Sinana district. The breeds of animals were the local zebu (predominant) and the zebu crossbred with Holstein-Friesian. The study animals consisted of 384 milking cows, 308 indigenous zebu, and 76 Holstein-zebu crosses, selected by simple random sampling method from smallholder dairy farms in chosen kebeles. All the study cows were hand milked and milked twice a day.

c) Study Design

A cross-sectional type of study supported by laboratory tests was carried out to determine the prevalence, major bacterial causes and to assess risk factors of bovine mastitis at the cow and quarter level from October 2013 to May 2014 on small holder dairy farms in and around Sinana district. Cows were examined directly at the quarter level for clinical manifestations and indirect tests (CMT) for subclinical mastitis.

d) Sampling Method and Determination of Sample Size

Sampling was accomplished using the simple random sampling technique to choose individual dairy cow. The sample size required for the study was calculated according to the formula given by Thrusfield (2007) for simple random sampling.

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

Where: n = required sample size,
Pexp = expected prevalence, and
d = desired absolute precision

Due to absence of logical research work undertaken in this district so far; the sample size is calculated using a technique suggested by Thrusfield (2007), with 95% confidence interval, at 5% desired absolute precision and expected prevalence of 50%. Hence; the total numbers of sample needed for this observation was 384 lactating dairy cows. Since the prevalence of mastitis was not known previously in the area, six kebeles (lowest administrative structure) were randomly selected using a lottery system out of the ten kebeles with a high number of dairy cows in the district. Proportionality of incorporating cattle in the sample will be applied as per the population size of each district and kebeles.

Table 1: Proportional allocation and number of animals sampled from each kebeles.

Kebeles	Number of Lactating Cows in the Kebeles	No. of Lactating Cows Sampled (Calculated Sample Size)
Basaso	2186	72
Nanno Robe	2058	66
Shallo	1855	61
Hora Boka	2102	69
Kabira Shaya	2339	77
Donsa	1150	39
Total	**11690**	**384**

Source: Data obtained from Sinana "Woreda" Agricultural Office (2013).

e) Sample collection and bacteriological examination

i. *Collection of milk samples*

Milk samples were collected according to the standard procedures recommended by National Mastitis Council NMC (2004). Approximately 10 ml of milk was collected aseptically from lactating cows into sterile test tubes after discarding the first three milking streams. Samples from each quarter were transported in the ice box (4°C) to Microbiology Laboratory of Debra Zeit School of Veterinary Medicine and Agriculture, where they were immediately cultured or stored at 4°C until processed or cultured on standard bacteriological media.

f) Examination of Clinical Mastitis

Clinical cases were recorded at the time of milk sampling. Clinical mastitis was diagnosed by the manifestation of visible signs of inflammation and abnormal milk. A quarter, which is warm, swollen and painful for the cow upon palpation was considered to have acute clinical mastitis; whereas atrophied, hard and fibrotic quarters were considered to have chronic mastitis (Quinn *et al.,*2004; Radostitits *et al.*, 2007).

g) California Mastitis Test screening

California Mastitis Test was performed for each quarter of a lactating cow. It is used to determine the prevalence of sub-clinical mastitis and also as the screening test for selection of samples to be cultured for the cows under study. A small sample of milk (approximately ½ teaspoon) from each quarter was collected into a plastic paddle that has four shallow cups marked A, B, C and D. An equal amount of California Mastitis Test reagent was added to the milk. The paddle was rotated to mix the contents. The CMT result was interpreted as negative (0), trace (T), weakly positive (+1), distinct positive (+2) and strongly positive (+3) as per the recommendation which is given by Quinn *et al*. (2004). Cows were considered positive for CMT when at least one quarter turned out to be positive for CMT. A herd was considered positive for CMT when at least one cow in a herd is tested positive for CMT.

h) Bacteriological examination of milk samples

i. *Cultural procedures and biochemical tests*

Isolation and identification of mastitis pathogens were conducted in the Microbiology Laboratory of Bishoftu, College of Veterinary Medicine and Agriculture. The bacteriological culture was executed following the standard microbiological techniques recommended by Quinn *et al.* (2004), National mastitis council (NMC) (2004). A loop full of milk was streaked on 5% sheep blood agar, nutrient agar, and MacConkey agar and then, the plates were incubated aerobically at 37 OC and examined after 24hrs of incubation for growth. The colonies were provisionally identified by staining reaction with Gram's stain, cellular morphology, colony morphology, pigmentation and hemolytic pattern on blood agar and other environment from which the bacterium was isolated. Subcultures were done to obtain pure isolates for further identification. In doing so, the representative colonies were subcultured on blood agar plate and nutrient slants and incubated at 37 OC. The slants were preserved and maintained for characterizing the isolates. Identification was done according to the standard methods described by Quinn *et al*. (2004).

i) Questionnaire survey of risk factors

Data was collected using a semi-structured questionnaire. The questionnaire was prepared, pre-tested and adjusted by translating into local language and administered by the same interviewer (researcher) who speaks the same language with the participant smallholders with the primary objective of elucidating the multifactorial background of mastitis. Data collected include intrinsic factors such as age, breed, parity, stage of lactation, previous history of mastitis and body condition. Extrinsic factors such as dry cow therapy, udder hygiene, drainage system, floor type and grazing system were also recorded.

j) Data Storage and Analysis

All data from laboratory tests and questionnaire were entered into a Microsoft Excel spreadsheet and accuracy was checked for statistical evaluation. After validation, data were transferred to STATA version 11.0 for Windows (Stata Corp. College Station, TX, USA) for analysis. The dependent variable suggested in the data analysis was mastitis status of a cow and the potential risk factors considered were parity of the cow, stage of lactation, breed, age, previous mastitis history and floor type. Prevalence was estimated as a percentage value. The relationship between the potential risk factors and the prevalence of mastitis was evaluated using the Chi-square test ($\chi 2$). Multivariate logistic regression analyses were used to analyze the effects of different supposed risk factors on the prevalence of mastitis. Odds ratio (OR) was utilized to determine the degree of association between putative risk factors with mastitis prevalence. The 95% confidence interval and a p-value <0.05 was considered statistically significant.

III. Results

a) Prevalence of mastitis

A total of 384 lactating cows (308 local and 76 crossbreed) were examined for mastitis detection. Out of the total examined, prevalence of mastitis at cow level was 36.72% (141/384), out of which 4.95% (19/384) and 31.77% (122/384) were clinical and sub clinical, respectively. A total of 1536 quarters were considered in this study and the quarter level prevalence was 26.43% (406/1536), from which 2.28% (35/1536) and 24.15% (371/1536) were found to be of clinical and subclinical forms, respectively (Table 2). Out of the 35 quarters with clinical cases, 1.30% (20/1536) was blind teats. The remaining, 0.98% (15/1536), was of a clinical form showing active cases of mastitis with manifested symptoms of inflammation on the udder and teat; and alterations in milk quality.

Table 2: Prevalence of mastitis at the cow and quarter level.

Forms of Mastitis	Total Numbers Examined	Total Numbers Affected (%)
Clinical		
Cow Level	384	19 (4.95)
Quarter Level	1536	35 (2.28)
Subclinical		
Cow Level	384	122 (31.77)
Quarter Level	1536	371 (24.15)
Overall		
Cow Level	384	141 (36.72)
Quarter Level	1536	406 (26.43)

In quarter level prevalence of subclinical mastitis, right rear teats (RR) showed the highest rate of infection (27.15%) followed by the left rear quarters (LR), 25.67%; left front teats (LF), 23.61% and the right front quarters (RF), 22.49% (Table 3).

Table 3: Quarter level prevalence of subclinical mastitis (Functional teats = 1501).

Quarter	No. Examined	Positive	Frequency (%)
RF	378	85	22.49
RR	372	101	27.15
LF	377	89	23.61
LR	374	96	25.67
Total	**1501**	**371**	**24.72**

RR, right rear; RF, right front; LR, left rear and LF, left front.

The number of lactating cows examined within each six study kebeles and percentages found to be positive for mastitis is depicted in Table 4. Mastitis prevalence in selected kebeles was highest in Donsa followed by Basaso, Nanno Robe, Hora Boka, Shallo and Kabira Shaya. There were no significant differences between the chosen kebeles of the investigated district and mastitis prevalence.

Table 4: Prevalence of bovine mastitis within the selected kebeles.

Sampled Kebeles	Number of Lactating Cows Examined	Number of Positive Cows	Prevalence (%)
Basaso	72	31	43.06
Nanno Robe	66	27	40.91
Shallo	61	16	26.23
Hora Boka	69	25	36.23
Kabira Shaya	77	19	24.68
Donsa	39	23	58.97
Total	**384**	**141**	**36.72**

b) Intrinsic risk factors associated with the prevalence of bovine mastitis

A Chi-square analysis revealed that prevalence of bovine mastitis was significantly associated with the age groups ($P<0.004$), parity ($P<0.05$), breed ($P<0.001$), stage of lactation ($P<0.001$), mastitis record ($P<0.001$) and udder hygiene ($P<0.01$). However, its association with body condition was not significantly varied ($P>0.05$) (Table 5).

Table 5: Chi-square analysis of intrinsic risk factors associated with the occurrence of mastitis.

Factor	Category	No. Examined	No. Positive	Prevalence (%)	$\chi 2$ (P Value)
Age	≤ 5 Years	134	36	26.87	
	> 5 Years	250	105	42.0	8.600 (0.003)
Parity	Primiparous	52	12	23.08	
	Multiparous	332	129	38.86	4.817 (0.028)
Breed	Local	308	89	28.89	
	Cross	76	52	68.42	40.984 (0.000)
Stage Of Lactation	Early (< 3 Months)	68	32	47.06	
	Mid (3–5 Months)	196	48	24.49	
	Late (> 5 Months)	120	61	50.83	26.032 (0.000)
Mastitis Record	No	331	112	33.84	
	Yes	53	29	54.72	8.572 (0.003)
Body Condition	Poor	146	56	38.36	0.970 (0.616)
	Medium	137	52	37.96	
	Good	101	33	32.67	

The results of logistic regression analysis of the association of different risk factors with the prevalence of bovine mastitis are depicted in Table 6. Analysis of the association of intrinsic risk factors with the prevalence using multivariable logistic regression showed that cross-breeds (OR=5.820, 95%CI: 3.248,10.430), early-stage lactation (OR = 3.021, 95%CI: 1.617, 5.647), late-stage lactating cows (OR=3.280, 95%CI: 1.931, 5.572) and previous mastitis record (OR=2.452, 95%CI: 1.282,4.688) were at higher risk of infection with bovine mastitis as compared to local breed, mid-stage lactation and non previous mastitis record, respectively.

Table 6: Multiple logistic regression analysis to predict the intrinsic risk factors associated with mastitis.

		Mastitis Test Tesult		Odds Ratio		
Factor	Category	No. Examined	No. Positive (%)	COR (95% CI)	AOR (95% CI)	P Value
Age	≤ 5 Years	134	36 (26.87)	1	1	
	> 5 Years	250	105 (42.0)	1.971 (1.248, 3.114)	1.784 (0.999, 3.189)	0.051
Parity	Primiparous	52	12 (23.08)	1	1	
	Multiparous	332	129 (38.86)	2.118 (1.071, 4.189)	1.320 (0.552, 3.155)	0.532
Breed	Local	308	89 (28.89)	1	1	
	Cross	76	52 (68.42)	5.331 (3.098, 9.175)	5.820 (3.248, 10.430)	0.000
Stage Of Lactation	Mid (3–5 Months)	196	48 (24.49)	1	1	
	Early (< 3 Months)	68	32 (47.06)	2.741 (1.539, 4.880)	3.021 (1.617, 5.647)	0.000
	Late (> 5 Months)	120	61 (50.83)	3.188 (1.965, 5.171)	3.280 (1.931, 5.572)	0.000
Mastitis Record	No	331	112 (33.84)	1	1	
	Yes	53	29 (54.72)	2.363 (1.314, 4.249)	2.452 (1.282, 4.688)	0.007

COR, Crude Odds Ratio; AOR, Adjusted Odds Ratio; CI, Confidence Interval; 1, Reference

c) *Extrinsic Risk Factors associated with the prevalence of bovine mastitis*

Management factors such as hygiene, dry cow therapy, housing, and grazing system were evaluated as extrinsic risk factors that influence the prevalence of bovine mastitis. The association between the occurrence of mastitis and extrinsic risk factors is presented in Table 7. Accordingly, mastitis prevalence showed significant variation with dry cow therapy (p = 0.021), udder/ teat hygiene (p = 0.001), drainage system (p= 0.033), floor type (p= 0.010) and grazing system (p=0.026).

Table 7: Chi-square analysis of extrinsic risk factors associated with the occurrence of mastitis.

Factor	Category	No. Examined	No. Positive	Prevalence (%)	$\chi 2$ (P Value)
Dry Cow Therapy	No	351	135	38.46	5.339(0.021)
	Yes	33	6	18.18	
Udder / Teat Hygiene	Poor	319	129	40.44	11.224(0.001)
	Good	65	12	18.46	
Drainage System	Poor	324	125	38.58	4.539(0.033)
	Good	64	16	25.00	
Floor Type	Soil	318	126	39.62	6.714(0.010)
	Concrete	66	15	22.73	
Grazing System	Zero Grazing	49	25	51.02	4.944(0.026)
	Grazing	335	116	34.63	

Risk factors logistic regression analyses showed that poor udder/teat hygiene had a significant effect (P<0.05) on the prevalence of mastitis. Bovine mastitis was more likely to occur in cows with poor udder/teat hygiene (OR = 13.386, 95%CI = 1.300, 137.845). Similarly, cows managed under zero grazing were more liable to mastitis (OR = 1.892, 95%CI = 1.022, 3.501) than cows under grazing. Odds of cows not receiving therapy during drying off was 1.445 times than those with dry cow therapy (Table 8).

Table 8: Multivariable logistic regression analysis of extrinsic risk factors associated with bovine mastitis.

		Mastitis Test Result	Odds Ratio		
Variable	Category	No. Positive (%)	COR (95% CI)	AOR (95% CI)	P Value
Dry Cow Therapy	No	135 (38.46)	2.812 (1.132, 6.990)	1.445 (0.467, 4.473)	0.523
	Yes	6 (18.18)	1	1	
Udder / Teat Hygiene	Poor	129 (40.44)	2.999 (1.542, 5.833)	13.386 (1.300, 137.845)	0.029
	Good	12 (18.46)	1	1	
Drainage System	Poor	125 (38.58)	1.923 (1.046, 3.535)	0.830 (0.323, 2.134)	0.698
Floor Type	Soil	126 (39.62)	2.231 (1.203, 4.139)	0.203 (0.022, 1.881)	0.161
	Concrete	15 (22.73)	1	1	
Grazing System	Zero Grazing	25 (51.02)	1.967 (1.075, 3.596)	1.892 (1.022, 3.501)	0.042
	Grazing	116 (34.63)	1	1	

COR, Crude Odds Ratio; AOR, Adjusted Odds Ratio; CI, Confidence Interval; 1, Reference.

d) *Bacterial Isolates*

From 343 positive culture samples, a total of 364 bacterial isolates were recovered. The most prevalent culture growth was *Staphylococcus aureus* (33.24%) followed by *Streptococcus agalactiae* (22.25%), *Staphylococcus epidermidis* (9.34%), *E.coli* (7.42%), Coagulase-Negative Staphylococci (CNS) (7.14%), *Streptococcus dysgalactiae* (5.77%),

Corynebacterium bovis (4.40%), *Streptococcus uberis* (3.85%), *Klebsiella pneumonia* (2.75%), *Pseudomonas aeruginosa* (2.2%) and *Bacillus cereus* (1.65%) (Table 9).

Table 9: Frequency and proportion of bacterial species isolated from bovine mastitis (number of isolates= 356).

Bacterial Species	Total Number of Isolates	Prevalence (%)
Staphylococcus Aureus	121	33.24
Streptococcus Agalactiae	81	22.25
Staphylococcus Epidermids	34	9.34
Escherichia Coli	27	7.42
Coagulase Negative Staphylococci	26	7.14
Streptococcus Dysgalactiae	21	5.77
Corynebacterium Bovis	16	4.40
Streptococcus Uberis	14	3.85
Klebsella Pneumonae	10	2.75
Pseudomonas Aeruginosa	8	2.20
Bacillus Cereus	6	1.65
Total	364	100.00

IV. Discussion

The present study revealed that the overall prevalence of bovine mastitis at cow level was 36.72%. This is comparable with the previous findings of Workineh *et al*. (2002), Biffa *et al*. (2005), and Abera *et al*. (2012) who reported 38.2% in Adami-Tulu in central Ethiopia, 34.9% in Southern Ethiopia, 37.1% in Shashemene in southern Ethiopia, respectively. However, the present finding is relatively lower than the report of Mungube *et al*. (2004), Sori *et al*. (2005), Bedada and Hiko (2011) and Bedane *et al*. (2012) who recorded 46.6% from central highlands of Ethiopia, 52.8% from Sebeta, 66.1% from Assela in south eastern Ethiopia, 59.1% from Yabello, southern Ethiopia, respectively. Moreover, Abdelrahim *et al.* (1990) found a prevalence of 45.8% in Sudan, Kivaria *et al*. (2004) reported a prevalence of 90.3% in Tanzania and Radostits *et al.* (2000) described the prevalence of mastitis to be around 50% in cows in most countries irrespective of the causative agent. On the other hand, the result of the present study is higher than the prevalence of 31.7% reported by Berhanu (1997) in Eastern Harerghe and 28.2% in Bahir Dar by Bitew *et al*. (2010). Mastitis is a complex disease, and the difference in the prevalence reports of mastitis in the present study and other reports could be attributable to differences in breeds of targeted cows, farm management practices, level of production and differences in study methods and materials employed by the investigators. The differences in prevalence are most likely due to individual cow factors that considerably influence mastitis prevalence (Mekonnen and Tesfaye, 2010).

The frequencies of clinical and subclinical mastitis are highly esteemed parameters in the evaluation of the health of the bovine mammary gland (Fonseca & Santos, 2001). The present study revealed that prevalence of clinical and sub clinical mastitis at cow level was 4.95% and 31.77%, respectively. This result is comparable with the finding of Benta & Habtamu (2011) and Moges *et al*. (2011) who reported 5.3% of clinical and 31.67% of subclinical mastitis at cow level, respectively. Moreover, Gizat *et al*. (2007) reported the prevalence of clinical and subclinical mastitis at the rate of 3.9 and 34.4%, respectively. However, higher prevalence rates of clinical mastitis (Kerro and Tareke, 2003 (37.1%); Almaw *et al*., 2009; (25.22%); Mekibib *et al*., 2010 (22.4%) and Bedane *et al*., 2012 (21.1%)) and subclinical mastitis (Kerro and Tareke, 2003 (62.9%); Mekibib *et al*., 2010 (48.6%); Benta & Habtamu, 2011 (46.6%) and Tesfaye *et al*., 2012 (41.4%)) has been reported. The difference in prevalence of subclinical mastitis may be due to the different husbandry practices, diagnostic techniques, environmental conditions and immune status of animals. Since, environmental factors play a significant role, the prevalence of clinical and subclinical mastitis varies in dairy animals (Radostits *et al*., 2007).

In this study subclinical mastitis has been found to be higher than clinical mastitis. This could be attributed to ease of detection of clinical mastitis and treatment of only clinical cases. In most developing countries including Ethiopia, the subclinical form of mastitis received little attention and efforts have been concentrated on the treatment of clinical cases (Aarestrup *et al*., 1994). Moreover, 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 (Hussein *et al*., 1997; Quinn *et al*., 2002; Mekonnen *et al*., 2005; Hundera *et al*., 2005). Because of its insidious nature, the subclinical mastitis might be among the causes of sub optimal milk production that is evident in many smallholder farms. According to Radostits *et al*. (2007), an infected cow and quarter show 30% and 15% reduction in milk yield, respectively. Moreover, farmers in Ethiopia are not well informed about the silent cases of mastitis (Karimuribo *et al*., 2006). Ethiopian farmers especially smallholders are not well informed about the invisible loss from sub clinical mastitis (Hussen *et al*., 1997) since dairying is mostly a side line business on these farms. A similar observation of the dominance of subclinical mastitis was observed by several studies (Workineh *et al*., 2002; Kerro and Tarek, 2003; Sori *et al*., 2011).

Overall quarter prevalence of 26.43% was recorded in the current study. The quarter prevalence of mastitis found in this study was comparable with the finding of Abera *et al*. (2010) in Adama, and Fadlelmoula *et al*. (2007) in Germany who reported the quarter

prevalence rate of 29% and 27.57%, respectively. However, the current report is lower than the report made by Mekibib *et al*. (2010) in Holeta, Bedane *et al*. (2012) in Yabello and Bachaya *et al*. (2011) in Pakistan, who reported 44.9%, 38.7%, and 35.25%, respectively. On the other hand, the present study is higher than the result of Kerro and Tareke (2003) from southern Ethiopia and Moges *et al*. (2011) from Gonder, who documented 18.7% and 12.73%, respectively. Quarter level prevalence of clinical (2.28%) and sub-clinical (24.15%) were observed which is in close agreement with the finding of Bitew *et al*. (2010) and Bedane *et al*. (2012) who recorded prevalence of clinical (1.9%) and subclinical (25.3%) mastitis at quarter level. However, it is lower than the previous report of Kerro and Tareke (2003) who reported the prevalence of clinical and subclinical mastitis to be 39.2, 60.8%, respectively. The difference in quarter wise prevalence of clinical and subclinical mastitis observed in the current study and previous studies may be due to the difference in breeds of animals, immune status, and managemental practices. The blind teat accounted 1.3%, which may be an indication of serious mastitis problem on the herd and lack of screening tests and treatment of subclinical mastitis, and inadequate follow up chronic mastitis were considered to be the major reason for the development of quarter blindness (Biffa, 2005). As compared to the others the right rear quarters were affected with the highest infection rate (27.15%). The left rear quarters were the second with an infection rate of 25.67%. This might be due to the high production capacity of the hind quarters followed with relaxed teat sphincters (Radostitis and Blood, 1994) and the high chance of getting fecal and environmental contamination (Sori *et al*., 2005). These results are supported by various other workers who also reported an increased prevalence of mastitis in rear quarters (Zeryehun *et al*., 2013; Zenebe *et al*., 2014).

The prevalence of mastitis was significantly associated with age and parity ($p < 0.05$). Thus, prevalence was relatively higher in adult cows (OR = 1.784), multiparous (OR = 1.320) than those corresponding animals. Significant association of age and parity with mastitis was reported by other authors (Abera *et al*., 2010; Moges *et al*., 2011; Zeryehun *et al*., 2013). Cows with many calves (>7) have about 13 times greater risk (62.9%) of developing an udder infection than those with fewer (3) calves (11.3%) (Biffa *et al*., 2005). The increased prevalence of mastitis in older animals in this study can be related to increased susceptibility of pathogenic organisms in udder relaxed sphincter muscles of teats. According to Erskine *et al*. (2002), primiparous cows have more effective defense mechanism than multiparous cows.

The prevalence of mastitis varied significantly ($p<0.001$) among breeds, where higher prevalence was recorded in the cross (68.42%) than Zebu (28.89%). Cross breed cows had shown to have a significant effect ($p<0.001$, OR=5.820, 95% CI = 3.248, 10.430) on the prevalence of bovine mastitis. The observed higher prevalence of mastitis in cross compared to local cows is in agreement with the findings of Biffa *et al*. (2005), Girma (2002) and Biru (1989). As stated in Radostits *et al*. (2007) this may be associated with differences in anatomical and physiological characteristics of the mammary gland, as well as high milk yielding of the cows. Furthermore, increase in milk yield from genetic selection may be accompanied in genetic susceptibility to mastitis. Therefore, the lower prevalence in local zebu cows in this study could be associated with the difference in genetic controlled physical barriers like streak canal sphincter muscle, keratin in the teat canal or shape of teat end where pointed teat ends are prone to the lesion. In addition to the physical barrier, the difference in the occurrence of mastitis in these breeds could arise from the difference in cellular immunity.

The finding of this study also showed the higher prevalence rate of mastitis in early (47.06%) and late (50.83%) stages of lactation as compared to mid (24.49%) stage of lactation with significant association ($p < 0.001$) with mastitis. Early and late-stage of lactation had shown to have a significant effect (early-stage, $p<0.001$, OR=3.021, 95% CI = 1.617, 5.647; late-stage, $P<0.001$, OR=3.280, 95% CI=1.931, 5.572) on the prevalence of bovine mastitis when compared to mid-lactation stage. This finding is in agreement with the previous results of Kerro and Tareke (2003) and Biffa *et al*. (2005) and Abera *et al*. (2012) who reported a high prevalence of mastitis in the early and late-stage of lactation. The udder is most sensitive to acute clinical mastitis and subclinical mastitis during the period after the calving, whereas chronic mastitis, most often subclinical, is more frequent later during the lactation. On the other hand, cows also get a natural high cell count towards the end of lactation because of reduced milk production (Andersson *et al*., 2011).

Cows with the previous history of mastitis had higher mastitis prevalence ($P<0.001$) compared to cows with no previous history of mastitis. The multiple logistic regression analysis also revealed a significant association of previous mastitis record (OR=2.452, 95%CI= 1.282, 4.688, $p<0.01$) with the prevalence of mastitis. Cows with the previous history of mastitis were found more likely to be mastitic. This observation is supported by the findings of Biffa *et al*. (2005) and Abera *et al*. (2012) who disclosed similar reports. This finding suggests that treatment of cows for mastitis may not be effective in eliminating the pathogens and the disease may be carried over from previous lactations to next lactation. Also, there are reports of antimicrobial resistance among pathogens which cause mastitis in Ethiopia (Abera *et al*., 2010).

Cows that were not treated during dry period were more affected than those treated and significantly associated with the prevalence of mastitis ($p<0.05$). This could be associated with the low bactericidal and bacteriostatic quality of milk during the dry period. Moreover, the capacity of the quarter to provide phagocytic and bactericidal activity generally diminishes during the dry period (Paape and Miller, 1996). Studies show that teat dipping after milking reduces the spread of infection from cow to cow, while dry cow therapy reduces the reservoir, which in turn further reduce the teats from bacterial exposure (Smith & Hogan, 1995). During the dry period, a keratin protein substance is produced to protect the streak canal (Eberthart, 1986).

The result of the present study also revealed the higher prevalence of mastitis (40.44%) in cows with poor udder/teat hygiene as compared to cows with good udder hygiene (18.46%). Odds ratio indicated that cows with poor udder hygiene were 13.39 times more likely to be exposed to mastitis than those with good udder hygiene. The current result is in agreement with the finding of Fentaye *et al*. (2014). Sanitary milking habits are important to avoid the spreading of bacteria or their proliferation. Milking practice had a significant influence on the prevalence of bovine mastitis. In this study, owners who didn't wash teats before and after milking found to have a high prevalence of mastitis than owners who used to. Improper washing of hands and teats before milking and use of one towel for each cow contribute to the prevalence of mastitis (Byarugaba *et al*., 2008). Radostitis *et al.* (2007) documented that udder preparation both before and after milking influence the rate of mastitis. Inadequate sanitation of dairy environment and lack of proper attention to the health of mammary gland were important factors contributing to the prevalence of mastitis (Musse *et al*., 2014).

Prevalence of mastitis was higher in those farms with poor drainage/slope for the stable area with significant association obtained between mastitis prevalence and drainage system which is in agreement with a report made by Abera *et al*. (2012). Poor drainage/slope of the stable area results accumulation of liquid such as urine and water used for cleaning of udders during milking. The liquid material mixed with the feces of the cows that led to dirty udder and teat. The environmental bacteria such as *E. coli* and other got access to enter trough teat canal and result in infection (Tesfaye *et al*., 2012).

Cows kept in houses with soil floor had a higher prevalence than cows managed on the concrete floor. Houses with soil floor increased the risk of mastitis. The association between soil floor and high prevalence of mastitis recorded in our study is consistent with the findings of Abera *et al*. (2010). This might be due to the favorable environment created for survival and multiplication of bacterial pathogens. Earlier works implicated poor barn hygiene to have a high prevalence of mastitis (Sori *et al*., 2005).

A significantly greater prevalence of mastitis was observed for cows maintained in zero grazing system (OR=1.892, 95%CI= 1.022, 3.501, $p<0.05$) than free grazers. Some authors affirmed that cows raised intensively are more susceptible to the development of intramammary infections through the greater concentration of animals and exposure to organic matter and pathogenic microorganisms (Kalmus *et al*., 2006).

The result obtained from bacteriological analysis of the samples revealed the predominant organisms isolated from bovine mastitis found to be *Staphylococcus aureus* (33.24%) followed by *Streptococcus agalactiae* (22.25%). *Staphylococci* and *Streptococci* species together accounted for 83.15% of the total isolates, while *Staphylococci* alone were 52.53% of the isolates. These bacteria were implicated as the most frequently isolated from mastitic milk in Ethiopia: *Staphylococci* and *Streptococci* species accounted for 73.5% (Workineh *et al*., 2002), 63.0% (Kerro and Tareke, 2003), 73.2% (Sori *et al*., 2005), 89.0% (Almaw *et al*., 2008), 57.2% (Mekonnen and Tesfaye, 2010) and 79.3% (Tesfaye *et al*., 2012) of the total isolates of bacteria from mastitic milk. The high prevalence of *Staphylococci* and *Streptococci* may be partly explained by presence of these agents on the skin and mucus membranes of various parts of the animal body (Carter and Wise, 2004; Quinn *et al*., 2004) and their contagious nature, especially *Staphylococcus aureus* and *Streptococcus agalactiae* (Radostits *et al*., 2007).

Moreover, the predominance and primary role of *Staphylococcus aureus* isolates in bovine mastitis has also been reported in other studies (Mekbib *et al*., 2010; Gitau *et al*., 2011; Asamenew *et al*., 2013; Alekish *et al*., 2013). Detection of *Staphylococcus aureus* at highest frequency in the current study could be due to its ability to evade and influence the host immune system by production of various enzymes and toxins that cause damage to mammary tissue and allow tissue invasion. In addition, *Staphylococcus aureus* is capable of surviving in the keratin of the teat canal of healthy cows and to confront phagocytosis. Furthermore, many *Staphylococcus aureus* strains can resist antibiotic therapy by the production of beta-lactamase, an enzyme that inactivates penicillin, and closely related antibiotics. Probably around 50% of mastitis caused by *Staphylococcus aureus* strains produce beta-lactamase and there is evidence that these strains are more difficult to cure with all antibiotics (Levy, 1998; Martin and Andrew, 2004). Furthermore, the finding of a higher proportion of *Staphylococcus* species might be due to lack of effective udder washing and drying, post-milking teat dip and drying and hand washing (Radostits *et al*., 1994). It is also attributed to the wide distribution of the

bacteria on the skin of teats and udder. The *staphylococci* have adapted to survive in the udder; they usually establish chronic, subclinical, infection and are shed in the milk which serves as a source of infection for other health cows during the milking process (Radostits *et al*., 2007).

In this study, *Streptococcus* species accounted for 31.87% of the total isolates next to *Staphylococcus* species. This finding was in agreement with Almaw *et al*. (2008), Mekonnen and Tesfaye, (2010) and Tesfaye *et al*. (2012). The relatively lower prevalence compared to *Staphylococcus* species might be due to their ready response to treatment as a cause of mastitis. The reason for the lower isolation rate of *Streptococcus* species is wide spread usage of penicillin for the treatment of mastitis because penicillin is effective antibiotic against this species of bacteria (Fantaye *et al*., 2014).

Coliforms (*Escherichia coli* and *Klebsiella pneumonia*) were the third most commonly isolated bacteria (10.17%) after *Staphylococci* and *Streptococci* which are in close agreement with the report of Kerro and Tareke (2003), Mekonnen and Tesfaye (2010) and Asamenew *et al*. (2013). Because these bacteria are environmental pathogens, their occurrence may be associated with poor quality management of housing, bedding and general lack of farm cleanliness and sanitation as they are commonly found in manure, soil and contaminated water (Hogeveen, 2005; Radostits *et al*., 2007).

The present study disclosed that prevalence of *Corynebacterium bovis* was 4.4% which was in close agreement with the report of Langoni *et al*. (2011). The natural habitat of *Corynebacterium bovis* is teat canal of cows (Quinn *et al*., 2004). Blowey and Edmondson (2010) reported the association of *Corynebacterium bovis* with poor post milking teat disinfection. Moreover, the current study revealed the prevalence of *Pseudomonas aeruginosa* at a rate of 2.2% that concord with the finding of Tesfaye *et al*. (2013). *Pseudomonas aeruginosa* is associated with contaminated water sources and can cause severe mastitis (Blowey and Edmondson, 2010).

V. Conclusion

The present study revealed that bovine mastitis is prevalent in smallholder dairy farms in the study area, and further confirms that the subclinical form is the most prevalent. The predominant bacterial species isolated in the study area were *Staphylococci* followed by *Streptococci species and* coliforms. Age, parity, breed, stage of lactation, previous mastitis record, udder hygiene, drainage/slope, floor type and grazing system were found to be risk factors significantly related to mastitis prevalence. Determination of mastitis causing organisms and putative potential risk factors is vital not only for the choice of treatment of the affected animals but also for devising effective management practices against associated risk factors. Bovine mastitis is prevalent in the study area and undoubtedly will hurt productivity of dairy industry and hence warrants serious attention. Regular screening for the detection of subclinical mastitis and proper treatment of the clinical cases, good milking hygiene as well as appropriate treatment of cows during dry and lactation period should be practiced.

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References Références Referencias

1. Abdelrahim A. I., Shommein A. M., Suliman H. B., Shaddard S. A. (1990). Prevalence of mastitis in imported Freisian cows in Sudan. Rev. Elev. Med. Vet. Pays. Trop. 42: 512-514.
2. Abera M., Elias B., Aragaw K., Denberga Y., Amenu K., and Sheferaw D. (2012). Major causes of mastitis and associated risk factors in small holder dairy cows in Shashemene, Southern Ethiopia. African Journal of Agricultural Research Vol. 7 (24), pp. 3513-3518.
3. Abera M., Demie B., Aragaw K., Regassa F., and Regassa A. (2010). 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* Vol. 2 (3), pp. 29-34.
4. Alekish M. O, Al-Qudah K. M., Al-Saleh A. (2013). Prevalence of antimicrobial resistance among bacterial pathogens isolated from bovine mastitis in northern Jordan. *Revue Méd. Vét.*, 164, 6, 319-326.
5. Ali, T., Rahman, A., Qureshi, M. S., Hussain, M. T., Khan, M. S., Din, S. U., Iqbal, M. and Han, B., (2014). Effect of management practices and animal age on incidence of mastitis in Nili Ravi buffaloes. *Trop. Anim. Hlth. Prod*., 46: 1279-1285.
6. Almaw G., W. Molla and A. Melaku (2009). Prevalence of bovine subclinical mastitis in Gondar town and surrounding areas, Ethiopia. Livestock Research for Rural Development 21 (7).
7. Andersson, I., Andersson, H., Christiansson, A., Lindmark-Månsson, H., Oskarsson, M., Persson, Y.

& Widell, A. (2011). Systemanalys Celltal. Stockholm: Svensk Mjölk Forskning. Nr. 7091.

8. Andrew, A. H., R. W. Blowey and R. G. Eddy, (2004). Bovine disease, medicine and husbandry of cattle. 2ND edition. Blackwell Science Ltd., pp: 326-360.
9. Awale M. M., Dudhatra G. B., Avinash K., Chauhan B. N., Kamani D. R., Modi C. M., Patel H. B., O'Kennedy R. (2012): Bovine mastitis: a threat to economy. Open Access Scientific Reports 1, 295. doi:10.4172/scientificreports.295.
10. Bachaya, H. A., M. A. Raza, S. Murtaza and I. U. R. Akbar (2011). Subclinical bovine mastitis in Muzaffar Garh district of Punjab (Pakistan). The J. Anim. Plant Sci. 21 (2):16-19.
11. Barkema H. W., Green M. J., Bradley A. J., Zadoks R. N. (2009): Invited review: the role of contagious disease in udder health. *Journal of Dairy Science* 92, 4717-4729.
12. Bedada, B. A. and A. Hiko (2011). Mastitis and antimicrobial susceptibility test at Asella, Oromia Regional state, Ethiopia. J. Microbiol. Antimicrobials, 3 (9), 228-232.
13. Bedane, A., G. Kasim, T. Yohannis, T. Habtamu, B. Asseged and B. Demelash, (2012). Study on Prevalence and Risk Factors of Bovine Mastitis in Borana Pastoral and Agro-Pastoral Settings of Yabello District, Borana Zone, Southern Ethiopia. American-Eurasian Journal of Agricultural and Environmental Science, 12 (10): 1274-1281.
14. Benta D. B., Habtamu T. M. (2011). Study on the prevalence of Mastis and its Associated Risk Factors in Lactating Dairy Cows in Batu and its Environs, Ethiopia. *Global Veterinaria*. 7 (6): 632-637.
15. Berhanu, S. (1997). Bovine mastitis in dairy farms in Dire Dawa and eastern Harerghe. Prevalence, isolation and in vitroantimicrobial susceptibility studies. DVM thesis, Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia.
16. Biffa D., Debela E., Beyene F. (2005). Prevalence and risk factors of mastitis in lactating dairy cows in Southern Ethiopia. Int. J. Appl. Res. Vet. Med. 3 (3): 189-198.
17. Biru, G. (1989): Major bacteria causing bovine mastitis and their sensativity to common antibiotic. *Ethiopia Journal of Agricultur Science*. 11: 47-54.
18. Bitew M., Tafere A., Tolosa T. (2010). Study on bovine mastitis in dairy farms of Bahir Dar. *Journal of animal and veterinary advances*, 9 (23): 2912-2917.
19. Blowey R. and Edmondson P. (2010). Mastitis Control in Dairy Herds, 2nd Edition. CAB International, Oxfordshire, UK, PP 177.
20. Bradley A. J. (2002): Bovine mastitis: an evolving disease. *Veterinary Journal* 164, 116–128.
21. Byarugaba, D. K., Nakavuma, J. L., Vaarst, M. & Laker, C., (2008). Mastitis occurrence and constraints to mastitis control in small holder dairy farming systems in Uganda, Livestock Research for Rural Development 20: 1.
22. Carter G. R., Wise D. J. (2004). Essentials of Veterinary Bacteriology and Mycology 6th edition. Blackwell Science Ltd, Iowa, pp. 465-475.
23. Eberthart, R. J., (1986). Management of dry cows to reduce mastitis. Journal of Dairy Science. 69, PP 1721-1732.
24. Erskine, R. J., Walker, R. D., Bolin, C. A., Bartlett, P. C. and White, D. G. (2002): Trends in antibacterial susceptibility of mastitis pathogens during a seven-year period. *J. Dairy Sci.,* 85: 1111-1118.
25. Fadlelmoula, A., R. D. Fahr, G. Anacker and H. H. Swalve (2007). The Management Practices Associated with Prevalence and Risk Factors of Mastitis in Large Scale Dairy Farms in Thuringia-Germany 1: Environmental Factors Associated with Prevalence of mastitis. Aust. J. Basic and Applied Sci. 1 (4): 619-624.
26. Fonseca L. F. & Santos M. V. (2001). Qualidade do Leite e Controle de Mastite. São Paulo, 72: 171-177.
27. Gillespie B. E., Headrick S. I., Boonyayatra S., Oliver S. P. (2009): Prevalence and persistence of coagulase-negative Staphylococcus species in three dairy research herds. *Veterinary Microbiology* 134, 65-72.
28. Girma, S., Mammo, A., Bogele, K., Sori, T., Tadesse, F. and Jibat, T. (2012). Study on prevalence of bovine mastitis and its major causative agents in West Harerghe zone, Doba district, Ethiopia. *J. Vet. Med. Anim. Hlth*., 4: 116-123.
29. Gizat, A., A. Zerihun and Y. Asfaw, (2007). Bovine mastitis and its association with selected risk factors in small holder dairy farms in and around Bahir Dar, Ethiopia. *Ethiopian Veterinary Journal*, 13: 23-26.
30. Gruet P., Maincent P., Berthelot X., Kaltsatos V. (2001): Bovine mastitis and intramammary drug delivery: review and perspectives. *Advanced Drug Delivery Reviews* 50, 245-259.
31. Hogeveen H. (2005). Mastitis in Dairy Production: Current Knowledge and Future Solutions 1st edition, 744 pages, Wageningen Academic Publishers, The Netherlands.
32. Hovinen M., Pyorala S. (2011): Invited review: udder health of dairy cows in automatic milking. *Journal of Dairy Science* 94, 547-562.
33. Hunderra S., Ademe Z., Sintayehu A. (2005). Dairy cattle mastitis in and around Sebeta, Ethiopia. Int. J. Appl. Res. Vet. Med. 3 (4): 34-37.
34. Hussen, N., T. Yehualashet and G. Tilahun, 1997. Prevalence of mastitis in different local and exotic breeds of milking cows. Ethiopian Journal of Agricultural Science, 16: 53-60.

35. Kalmus P., Viltrop A., Aasmäe B. & Kask K. (2006). Occurrence of clinical mastitis in primiparous Estonian dairy cows in different housing conditions. *Acta Vet. Scand*. 48: 21.
36. Karimuribo, E. D., J. L. Fitzpatrick, C. E. Bell, E. S. Swai, D. M. Kambarage, N. H. Ogden, M. J. Bryant and N. P. French (2006). Clinical and subclinical mastitis in small holder dairy farms in Tanzania: Risk, intervention and knowledge transfer. Prev. Vet. Med. 74: 84-98.
37. Kerro, O. and Tareke, F. (2003): Bovine mastitis in selected areas of Souther Ethiopia. *Trop. Anim. Health and Prod.,* 35: 197-205.
38. Kivaria, F. M., Noordhuizenm, J. P. T. M. & Kapaga, A. M., (2004). Risk indicators associated with subclinical mastitis in Small holder Dairy cows in Tanzania. Tropical Animal Health and Production 36, pp. 581-592.
39. Langoni H., Penachio D. S., Citadella J. C. C., Laurino F., Faccioli-Martins P. Y., Lucheis S. B., Menozzi B. D. & Silva A. V. (2011). Aspectos microbiológicos e de qualidade do leite bovino. Pesq. Vet. Bras. 31:1059-1065.
40. Lemma, M., T. Kassa and A. Tegegene, (2001). Clinically manifested major health problems of crossbred dairy herds in urban and periurban production system in the central highlands of Ethiopia. Tropical Animal Health and Production, 33: 85-89.
41. Levy., S. (1998). The challenge of antibiotic resistance. *Sci. Am*., 278, 46–53.
42. Martin Green, Andrew Bradley (2004). Clinical Forum - *Staphylococcus aureus* mastitis in cattle. Cattle_Practice. Uk. Vet., Vol. 9, No 4, July. http://ovg.co.uk/Staph%20aureus%20mastitis%20 in %20cattle.pdf.
43. Mekibib B., Furgasa M., Abunna F., Megersa B., Regassa A. (2010). Bovine mastitis: prevalence, risk factors and major pathogens in dairy farms of Holeta town, central Ethiopia. *Vet. World* 13 (9): 397-403.
44. Mekonnen H., Workineh S., Bayleyegne M., Moges A., Tadele K. (2005). Antimicrobial susceptibility profile of mastitis isolates from cows in three major Ethiopian dairies. Med. Vet. 176 (7): 391-394.
45. Mekonnen H. and Tesfaye A. (2010). Prevalence and etiology of mastitis and related management factors in market oriented small holder dairy farms in Adama, Ethiopia. *Revue Méd. Vét*., 161, 12, 574-579.
46. Moges N., Yilikal Asfaw and Kelay Belihu (2011). A Cross Sectional Study on the Prevalence of Sub Clinical Mastitis and Associated Risk Factors in and Aronund Gondar, Northern Ethiopia. *International Journal of Animal and Veterinary Advances* 3 (6): 455-459.
47. Mungube E. O., Tenhgen B. A., Kassa T., Regassa F., Kyule M. N., Griener M., Baumann M. P. O. (2004). Risk factor for dairy cow mastitis in the central highlands of Ethiopia. *Trop. Anim. Hlth. Prod.*, 36, 463-467.
48. Musse T., Tesfu K., Dawit G. and Temesgen M. (2014). The Occurrence of Bovine Mastitis and Associated Risk Factors in and Around Addis Ababa, Central Ethiopia. *Applied Journal of Hygiene* 3 (3): 45-50.
49. N. M. C. (2004). National Mastitis Council: Microbiological procedures for the diagnosis of bovine udder infection. National Mastitis Council Inc., 3rd edition, Madison, Wisconsin, PP 7-8.
50. Oliver S. P. & Murinda S. E. 2012. Antimicrobial resistance of mastitis pathogens. Vet. Clin. *North. Am., Food Anim. Pract*. 28: 165-185.
51. Quinn, P. J., B. K. Markey, M. E. Carter, W. J. Donelly and F. C. Leonard, 2002. Bacterial of bovine mastitis. Veterinary microbiology and microbial diseases. Blackwell Science Ltd, Blackwell Publishing Company, PP: 465-475.
52. Quinn, P. J., Carter, M. E., Markey, B. K. and Carter, G. R. (2004): Mastitis. In: Clinical Veterinary Microbiology, Mosby International Limited, London, PP 327-344.
53. Paape, M. J. and R. M. Miller (1996). Influence of involution on intramammary phagocytic defense mechanism. J. Dairy Sci. 1992:56.
54. Radostitis O. M., Blood D. C., Gay C. C. (1994). Veterinary Medicine: A text book of the diseases of cattle, sheep, pigs, goats and horses. 8th edition. Bailliere Tindall: London 8: 563-613.
55. Radostits, D. M., D. C. Blood and C. C. Gay, (1994). Veterinary Medicine: A Textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses. 8th edition. Bailliere Tindall: London, UK. PP: 501-550.
56. Radostits O. M., GAY G. C., Blood D. C., Hinchillif K. W. (2000). Mastitis In: Veterinary Medicine, 9th Edition, Harcourt Limited, London PP. 603-700.
57. Radostits O. M., Gay C. C., Blood D. C., Hinchllif K. W. (2007). Mastitis. In: Veterinary Medicine 9th edition., Haracourt Ltd, London, PP. 174-758.
58. Rall, V. L. M., E. S. Miranda, I. G. Castilho and C. H. Camargo, (2013). Diversity of Staphylococcus species and prevalence of enterotoxin genes isolated from milk of healthy cows and cows with subclinical mastitis. J. Dairy Sci., 97: 829-837. DOI: 10.3168/jds.2013-7226.
59. Rysanek D., Babak V., Zouharova M. (2007): Bulk tank milk somatic cell count and sources of raw milk contamination with mastitis pathogens. *Veterinarni Medicina* 52, 223-230.
60. Schroeder J. (2012). Bovine Mastitis and Milking Management. North Dakota State University.

Available: www.ag.ndsu.edu/pubs/ansci/dairy/as1129.pdf.

61. Schukken, Y. H., Wilson, D. J., Welcome, F, Garrison-Tikofsky, L. and Gonzalez, R. N. (2003): Monitoring udder health and milk quality using somatic cell counts. *Vet. Res.,* 34: 579-96.
62. SWARDO (2013). Sinana Woreda Agricultural and Rural Development Office.
63. Smith, K. L. & Hogan, J. S., (1995). Epidemiology of Mastitis. In Third IDF international mastitis seminar, Lachmann Printers Ltd. Haifa, Israel. PP. 82-83.
64. Sori, H., Zerihun, A., Abdicho, S. (2005): Dairy cattle mastitis in and around Sebeta. *Intern J Appl Res Vet Med.* 3: 338-341.
65. Sori, T., J. Hussien and M. Bitew (2011). Prevalence and susceptibility assay of *Staphylococcus aureus* isolated from bovine mastitis in Dairy Farms of Jimma Town, South West Ethiopia. J. Anim. Vet. Adv. 10 (6): 745-749.
66. Steeneveld, W., Hogeveen, H., Barkema, H. W., Broek, V. D. and Huirne, B. M. (2008). The influence of cow factors on the incidence of clinical mastitis in dairy cows. J. Dairy Sci., 91: 1391-1402.
67. Suriyathaporn, W., Y. H. Suchkken, M. Nielen and A. Brands, (2000). Low somatic cell count: A risk factor for subsequent clinical mastitis in dairy herds. *Journal of Dairy Science*, 83: 1248-1255.
68. Teklemariam D. A., Ashebir G., Tassew A., Biruk T., Aklilu F., Tesfaye S. (2016). Isolation and phenotypic characterization of coagulase negative staphylococcus isolated from mastitic Cows in and around Zway town, Ethiopia. *International Journal of Fauna and Biological Studies.* 3(1): 45-54.
69. Tesfaye A., Aster Y., Addisalem H., Tegegnework T. and Zelalem G. (2013). Mastits: Prevalence, risk factors and antimicrobial sensitivity patterns of bacterial isolates in dairy cattle at Holeta farm in Ethiopia. *African Journal of Agricultural Research.* Vol . 8 (23), PP. 2837-2842.
70. Tilahun A., Aylate A. (2015). Prevalence of Bovine Mastitis in Lactating Cowsand its Public Health Implications in Selected Commercial Dairy Farms of Addis Ababa. Global Journal of Medical Research: *G Veterinary Science and Veterinary Medicine*, Volume 15 Issue 2 Version 1.0.
71. Tiwari, J. G., Babra, C., Tiwari, H. K., Williams, V., Wet, S. D., Gibson, J., Paxman, A., Morgan, E., Costantino, P., Sunagar, R., I. sloor, S. and Mukkur, T. (2013). Trends in therapeutic and prevention strategies for management of bovine mastitis: An overview. J. Vaccines Vaccin, 4: 1000176.
72. Watts J. L. (1988): Etiological agents of bovine mastitis. *Veterinary Microbiology* 16, 41-66.
73. Wilson, D. J., Gonzalze, R. N., Das, H. H. (1997): Bovine mastitis pathogens in New York and Pennsyluania: Prevalence and effect on somatic cell count and milk production. J. Dairy Sci. 80: 2592-2598.
74. Workneh S., Bayleyegne M., Mekonen H., Potgreter L. (2002). Prevalence and etiology of mastitis in cow from two major Ethiopian dairies. Trop. Anim. Prod. 34:19-25.
75. Zenebe, N., H. abtamu, T. and Endale, B., (2014). Study on bovine mastitis and associated risk factors in Adigrat, Northern Ethiopia. *Afr. J. Microbiol. Res*., 8: 327-331.
76. Zeryehun T., Aya T., Bayecha R. (2013). Study on prevalence, bacterial pathogens and associated risk factors of bovine mastitis in smallholder dairy farms in and around Addis Ababa. Ethiopia. *J. Anim. Plant Sci*., 23 (1): 50-55.
77. Zhao X., Lacasse P. (2007). Mammary tissue damage during bovine mastitis, cause and control. J. Animal Sci., 86: 57-65.