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Introduction- Ethiopia is believed to have the largest livestock population in Africa. This livestock sector has been contributing considerable portion to the economy of the country, and still promising to rally round the economic development of the country. It is eminent that livestock products and by-products in the form of meat, milk, honey, eggs, cheese, and butter supply provide mainly the needed animal protein that contributes to the improvement of the nutritional status of the people (CSA, 2009). Even though Ethiopia is the most populous country in cattle than any African country; the per capita milk consumption was 16 kg, which was lower than other countries in the region (Asfaw, 1997). This is partly due to the low genetic milk production potential of the indigenous zebu cattle. To increase milk production cross breeding of indigenous zebu with exotic breeds particularly with Holstein Friesian is widely practiced which resulted in a larger portion of the dairy cattle population especially in urban areas to be with a high level of exotic blood. However, this market oriented dairy production, a rapidly growing system in many African countries, is subjected to diseases of intensification including mastitis and reproductive disorders (Lemma *et al.*, 2001).

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A Study on the Prevalence of Bovine Mastitis and Associated Risk Factors in and the Surrounding areas of Sodo Town, Wolaita Zone, Ethiopia

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I. INTRODUCTION

Ethiopia is believed to have the largest livestock population in Africa. This livestock sector has been contributing considerable portion to the economy of the country, and still promising to rally round the economic development of the country. It is eminent that livestock products and by-products in the form of meat, milk, honey, eggs, cheese, and butter supply provide mainly the needed animal protein that contributes to the improvement of the nutritional status of the people (CSA, 2009). Even though Ethiopia is the most populous country in cattle than any African country; the per capita milk consumption was 16 kg, which was lower than other countries in the region (Asfaw, 1997). This is partly due to the low genetic milk production potential of the indigenous zebu cattle. To increase milk production cross breeding of indigenous zebu with exotic breeds particularly with Holstein Friesian is widely practiced which resulted in a larger portion of the dairy cattle population especially in urban areas to be with a high level of exotic blood. However, this market oriented dairy production, a rapidly growing system in many African countries, is subjected to diseases of intensification including mastitis and reproductive disorders (Lemma *et al.*, 2001).

Ethiopia holds large potential for dairy development due to its large cattle population and the favorable climate for improved high yielding animal breeds (Bishi, 1998). Considering the potential if smallholder income and employment generation, development of dairy farming can make significant contribution to the poverty reduction and nutritional improvement in the country (Staal, 1996). Dairy production is a biological efficient system that converts large quantities of roughage which is the most abundant of feed to milk (Reugg, 2001). In Ethiopia, where access to market dairying is preferred to meet production since it makes more efficient use of feed resource and

provides a regular income to the producers. Milk is very nutritional food that is rich in carbohydrate, protein, fat, vitamin and minerals. The increase in human populations, accessibility to technology input and high demand for animal product purchasing power in urban center had helped the urban and per-urban dairy farm in the country to flourish (Yoseph *et al.*, 1998).

Mastitis is one of the most important disease affecting dairy cows and it is a multi-factorial disease with worldwide distribution which incurs serious economic losses to dairy industry (DeGrave and Fetrow, 1993). A number of previous reports from different part of Ethiopia indicated that mastitis is a serious problem in dairy industry (Bishi, 1998). Bovine mastitis can reduce milk yield, increase culling rate, incur treatment cost, and occasionally result in death from severe infection (Radostitis *et al.*, 2007). Moreover, mastitis had been known to cause a great deal of loss or reduction of productivity, to influence the quality and quantity of milk yield, and to cause culling of animals at an unacceptable age (Singh and Singh, 1994). Generally, as with most infectious disease, mastitis risk factors depends on three components that is exposure to the microbes, cow defense mechanism, and environment and management factors (Suriyasathaporn *et al.*, 2000). Therefore, the objectives of this study were to determine the prevalence of bovine subclinical mastitis, to identify the major bacteria that cause subclinical mastitis and to determine the various risk factors associated with the occurrence of mastitis in and surrounding areas of Sodo town, Wolaita zone, Ethiopia.

II. MATERIALS AND METHODS

a) Study area

The study was carried out in small and large scale dairy farms in Sodo town and the surroundings, Southern Ethiopia. Sodo town is located about 329 km south of Addis Ababa at an altitude of 700-2950m above sea level. Sodo town is administrative center of Wolaita zone. The zone has an average annual rain fall ranging from 450-1446 mm. The rain fall over much of the areas is typically bimodal with the major rainy season extending from June-September and the short rainy

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season occurs from February-April. The mean annual maximum and minimum temperature of the area is 34.12 and 11.4°C, respectively (SZPEDD, 2001). The livestock population of Wolaita zone is estimated to be 886,242 bovine, 117,274 ovine, 99,817 caprine, 41,603 equines and 442,428 poultry (WZFEDD, 2005).

b) Study population

The study populations constituting lactating local (indigenous zebu), Holstein-Friesian breed, Holstein Friesian cross with local zebu breed and Jersey breed with no visually observed mastitis clinical sign and those are found in small and large scale dairy farms of Sodo town and the surroundings.

c) Study design

A cross sectional study design in which all the study animals were seen visually for non-clinical mastitis by physical examination of the udder and then tested for subclinical mastitis by CMT (California Mastitis Test). Information regarding the potential risk factors such as age, parity, stage of lactation, frequency of milking and hygiene of the farm were collected by questioner survey and by the observation of the investigators.

d) Sample size

The sample size for the study was calculated based on the formula developed by Thrust field (2005) for random sampling method. A 5% absolute precision and 95% confidence interval is used for determining sample size. Since there is a previous study on the prevalence of mastitis in the study area, an expected prevalence of 29.5% is used to determine the maximum sample size.

$$N = \frac{1.96^2 \times P \exp (1-P \exp)}{d^2}$$

Where N = the total sample size

Pexp = expected prevalence

d = absolute precision. Therefore, the calculated sample sizes was 319 samples

e) Sampling method

Strict aseptic procedure was followed when collecting milk samples to prevent contamination with microorganisms present on the skin of udder and teats, on the hands of samplers and barn environment. Teat ends were cleaned and disinfected before sampling. Strict foremilk (first jets) were discharged to reduce the number of contamination of teat canal. Sterile universal bottle with tight fitting cups were used. The universal bottle was labeled with permanent marker before sampling. To reduce contamination of teat ends during sample collection, the near teats were sampled first and then followed by the far ones (Quinn *et al.*, 1999).

f) Study methodology

i. Physical examination of the udder and milk

The udders were first examined visually and then palpated to detect any possible fibrosis,

inflammatory swelling and atrophy of the tissue. The size and consistency of the mammary quarter was inspected for the presence of any abnormalities such as disproportional symmetry, swelling, firmness and blindness of the teat canal. In addition, two streaks of milk from each quarter in a strip cup was inspected by visual inspection for presence of any flakes, clots, pus, watery appearance, blood and color change.

g) California Mastitis Test

After physical examination and strict aseptic procedure followed clinically free of mastitis cow was first tested by California mastitis test (CMT). Subclinical mastitis was diagnosed based on CMT result and the nature of coagulation and viscosity of the mixture, which show the presence, and the severity of the infection, respectively (Radostits *et al.*, 1994). CMT grades were evaluated and the result was scored based on the gel formation and categorized as negative if there was no gel formation, or positive if there was as 0 and 1 for negative and 2 and 3 for positive (Kerro Dego and Tareke, 2003). Milk samples were collected from each sub clinically mastitic non-blind quarters of the CMT positives cows for bacterial isolation. Then, 5ml positive milk sample was collected by sterile universal bottle from CMT positive quarter and transported to Wolaita Sodo Regional Veterinary Laboratory for further examination. When immediate inoculation was not convenient, samples was stored at 4°C until cultured for isolation.

h) Laboratory work

i. Culturing and Biochemical tests

Loop of milk sample was streaked on 5% sheep blood agar and plates were incubated aerobically at 37°C and examined after 24hrs of incubation for growth. The colonies were provisionally identified on the basis of staining reaction with Gram's stain, cellular morphology and hemolytic pattern on blood agar. The representative colonies were sub cultured on blood agar plate and on nutrient slants and incubated. The slants were preserved and maintained for characterizing the isolates slide Catalase tests, KOH and IMVIC tests for further isolation.

j) Data Management (Statistics)

Data was coded and entered to MS Excel spreadsheet and checked for accuracy. Pearson's chi-square test or Fisher's exact test was used to analyze the proportions of categorical data. Information regarding the potential risk factors for sub clinical mastitis such as age, breed, parity and hygienic management of the farm was analyzed.

III. RESULTS AND DISCUSSION

The present study was carried out to determine the prevalence of bovine subclinical mastitis, to identify the major bacteria that cause mastitis and to determine the various risk factors associated with the occurrence

of mastitis in and surrounding areas of Sodo town, Wolaita zone, Ethiopia. Of 319 samples collected from small scale dairy farms of the study area and were screened by CMT, which yielded an overall prevalence of 32.92% that is 105 animals examined had infection in their udders as evidence of mastitis. Findings of the present study closely agree with those of M.A.Islam *et al.*, (2011) who reported 29%. This study prevalence was lower than the findings of Lidet *et al.*, (2013) who reported 58%.

a) Breed related prevalence

Breed difference can play a vital role in the prevalence of different animal diseases. In this study area, four different breeds of cows are there especially at dairy farm level. The finding of this study was assessed for breed predisposition to the prevalence of SCM among the four breeds namely: Indigenous zebu, Holstein, Jersey and Holstein Friesian cross.

Accordingly, highest prevalence of SCM was revealed in Jersey (62.5%) and Holstein Friesian (47.5%), while lowest was recorded in Indigenous zebu (16.67%) and Holstein Friesian cross (16.78%) as shown in table-1 below. The highest prevalence observed both in Jersey and Holstein Friesian was statistically significant ($P < 0.05$). It has been reported that mastitis prevalence may be influenced by some inheritable characteristic such as capacity of milk production teat characteristic and udder conformation (Abaineh, 1997). However, the insignificant difference in the prevalence of mastitis between Jersey and Holstein Friesian as well as Indigenous zebu and Holstein Friesian cross reported in this work needs further investigation. It is worthwhile to mention here that the indigenous zebu and their crosses stocks are subjected to poor management conditions as compared to Jersey and Holstein cows.

Table-1 : The prevalence of mastitis by breed

Breed	Positive No. (%)	Negative No. (%)	χ^2 ; P-value
Jersey	20 (62.5%)	12 (37.5%)	$\chi^2 =$ $P =$
Indigenous zebu	3 (16.67%)	15 (83.33%)	
Holstein Friesian	57 (47.5%)	63 (52.5%)	
Holstein Friesian cross	25 (16.78%)	124 (83.22%)	
Total	105 (32.92%)	214 (67.08%)	

b) Different age group based prevalence

Age is a detrimental factor in the distribution of various diseases because at some time it is stressor. Hence, in the present study it was taken into consideration and the prevalence of mastitis was measured for different age groups of lactating cows.

The prevalence of subclinical mastitis was recorded as 69.8%, 33.5% and 34.15% at the age group of <4years, 5-7 years and >7 years, respectively. The prevalence was found to be much higher in the young than both the adult and older age group (table-2). This is actually found to be statistically significant with $P < 0.05$.

Table-2 : The prevalence of mastitis with respect to different age group

Age group	Positive (%)	Negative (%)	Total (%)	P-value
<4 years	19 (69.8%)	44 (30.2%)	63 (19.75%)	$P = 0.00$
5-7 years	72 (33.5%)	143 (66.5%)	215 (67.40%)	
>7 years	14 (34.15%)	27 (65.85%)	41 (12.85%)	
Total	105	214	319	

c) Parity related prevalence

The prevalence of varies livestock infection generally increases with increasing lactation number. The finding of this study was also assessed for the number of parity as predisposition to mastitis (table-3). Accordingly, in this study it indicates that the prevalence of subclinical mastitis was found highest both at ≤ 2 parity (43.33%) and ≥ 5 parity (43.33%) in comparison to

3rd and 4th parity and it was showed statistically significant variation with $P < 0.05$. But increasing tendency with prevalence of SCM was recorded with increase of parity and this observation supports with the reports of Rassl *et al.*, (1985) and Devi *et al.*, (1997) both of them reported an increasing prevalence of SCM with advancing parity.

Table-3 : Showing prevalence of mastitis in different parity groups of animals

Parity group	No. of animals examined		Prevalence (%)	P-value
	Affected (%)	Non-affected (%)	Total	
≤ 2	26 (43.33%)	34 (56.67%)	60 (18.81%)	$P = 0.000$
3-4	59 (27.7%)	154 (72.3%)	213 (66.77%)	
≥ 5	20 (43.48%)	26 (56.52%)	46 (14.42%)	
Total	105	214	319	

d) Prevalence of mastitis based on milking hygiene

Several factors in the environment affect the exposure of a cow to microorganisms. Sources of environmental exposure are manure, bedding, feeds, dirt, mud and water. A good example of this is *E.coli*, which is present in the environment of the cow. Several studies have indeed linked the cleanliness of the barn, and the colony count in the bedding with the incidence of clinical mastitis (Bramley and Neave 1975). Of critical importance is hygiene in the dry period. Most infections with coliform and environmental streptococci take place in the last two weeks before calving, and often only show signs of clinical mastitis after calving. Reducing exposure of the mammary gland by improving hygiene or providing a physical barrier at the teat end have shown to reduce the incidence of infections in this period.

e) Isolation of bacteria from sub clinical mastitis cases

In the present study, mastitis causing bacteria were isolated from sub clinical mastitis cases. Among the bacterial, *Staphylococcus* species 60 (57.14%) dominated followed by *Sterptococcus* species 30 (28.57%) and *E.coli* 15 (14.29%) which was isolated from sub clinical mastitis cows. With regard to the bacteriological analysis of milk sample, the work revealed that from the CMT positive milk sample the mixed bacterial isolates were the most prevalent than each isolated bacteria. It was reported that *Streptococcus* species together with *Staphylococcus* species were the most important causes of bovine mastitis (Blood and Radostitis, 1989). And the species of bacteria isolated *S.aureus* was most commonly isolated in sub clinical case of mastitis in this study case. The high level isolation of *Staphylococcus* species in this study is related with the finding of Ahamed and Mohammed (2007) in Egypt. This finding was not in harmony with reports of Bishi (1998) and Edwards *et al.* (1982) who found CNS as the predominant species from urban and peri-urban production system in Ethiopia and Bolivia, respectively. The reason for the higher isolation rate of this organism is the wide ecological distribution inside the mammary gland and skin. In area where hand milking and improper use of drug is practiced to treat the mastitis cases, its domination has been reported by many research scholars. *S.aureus* is adapted to survive in the udder and usually establishes mild sub clinical infection of long duration from which it is shaded through milk serving as sources of infection for other healthy cows and transmitted during the milking process (Radostitis *et al.*, 1994). Hence, the organism has been assuming a position of major importance as a cause of bovine mastitis.

The finding was also slightly in agreed with the findings of Molalegne *et al.* (2010) and Mengistu (1986) who reported *E.coli* species with the infection rate in this study was lower as compared to the other bacterial

species. In general, the prevalence of mastitis causing agents is high in subclinical cases. Thus, the farms should follow the key factors of mastitis program such as good herd management, teat dipping before and after milking, washing milkers hands before and after milking, preparation of clean towel for each lactating cow, milking of infected cow lastly, using dry cow therapy method and treating clinical cases at early stage.

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