

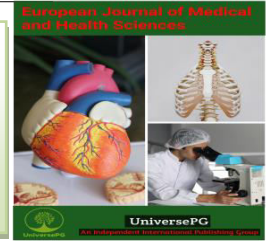


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Prevalence of Subclinical Mastitis among Small Ruminants and Isolation of Some Etiological Bacterial Pathogens in Jimma Town, Ethiopia

Wakgari Abirham Hayle^{1*}, Rokeya Ahmed² and Md. Ekhlash Uddin³

¹College Veterinary Medicine and Animal Sciences, University of Gondar, Ethiopia; ²Dept. of Veterinary & Animal Sciences, Gono Bishwabidyalay, Dhaka, Bangladesh and ³Dept. of Biochemistry & Molecular Biology, Gono Bishwabidyalay, Dhaka, Bangladesh.

*Correspondence: bashirabirham@gmail.com

ABSTRACT

Subclinical Mastitis in small ruminants is of concern due to the animal welfare, economic, public health, productivity, and livelihood impacts that it may pose. A cross-sectional study was conducted from October 2017 to April 2018 in and around Jimma town, Southwest, Ethiopia; to estimate the prevalence of subclinical mastitis, to isolate bacterial pathogens responsible for the occurrence of subclinical mastitis, and to identify risk factors associated with the development of subclinical mastitis in small ruminants. Milk samples were collected from 186 apparently healthy lactating small ruminants, tested on the field with the California Mastitis Test, and samples tested positive were subjected to bacteriological examinations. Out of 372 milk samples collected, 92(24.8%) samples from 70 animals were positive by the California Mastitis Test and these were cultured on different media. Through bacteriological examinations, six isolates of bacteria were identified which include; *S. aureus* (8.1%), *S. epidermidis* (10.2%), *S. intermidus* (2.2%), *S. hyicus* (1.6%), *S. agalactiae* (3.2%), and *E. coli* (12.4%). The highest prevalence of subclinical mastitis recorded was due to *Staphylococcus* species (22%) followed by *E. coli* (12.4%) and the least prevalence was due to *S. agalactiae* (3.2%). The overall prevalence of subclinical mastitis among small ruminants was 37.6%. Age and parity number variations among small ruminants were found important in influencing the prevalence in statistically significant ($P = 0.00$) extents. The highest prevalence of subclinical mastitis was recorded in old (24.7%) and in small ruminants having >5 parity numbers (24.7%). The high prevalence of small ruminant subclinical mastitis in the study area suggests that intervention strategies their-goal-being prevention and control of subclinical mastitis should be designed; so as to improve the welfare of animals, to save people consuming the milk of small ruminants from zoonotic risks, and to harvest the diverse benefits that may be generated from small ruminant production.

Keywords: California Mastitis test, Prevalence, Subclinical mastitis, Small ruminants, and Zoonotic diseases.

1. INTRODUCTION:

Small ruminant production supplies wide range of products and services such as milk, meat, wool, hide and skin, immediate income generation and associated social functions for poor farmers in many parts of Ethiopia (Adane and Girma, 2008). Sheep and goats

with their higher reproduction capacity, rapid growth rate, and their ability to give production in small and harsh environments are ideally suiting to produce by resource-poor smallholders (Tibbo, 2006).

Despite the huge sheep and goat population leading African countries in Ethiopia, small ruminant

production and productivity and producers' benefits remain far below to the expectation due to continuously occurring production constraints such as disease, malnutrition, poor animal production system, reproductive inefficiency, management constraints, and general lack of veterinary care. Among these factors, disease takes great portions and could be caused by infectious and non-infectious agents (Tibbo *et al.*, 2003). In dairy industry, udder health problem is one of the major diseases posing negative impacts on milk production, milk quality and animal welfare (Seegers *et al.*, 2003). Among udder health problems, mastitis constitutes one of the major problems and is defined as an inflammation of the mammary glands characterized by changes in the physical and chemical features of milk and pathological changes in the glandular tissue often with non-specific and complex factors responsible for its occurrence (Radostits *et al.*, 2007). It occurs worldwide in dairy animals and it has been described to have an extreme zoonotic and economic impact (AlMajali *et al.*, 2008). Mastitis occurs in two forms in small ruminants namely: clinical mastitis and subclinical mastitis. Clinical mastitis occurs with the evidence of milk and udder changes. Subclinical mastitis could be only detected by indirect tests such as CMT, WST, milk SCC and pathogen isolation at laboratory (Ekhlis *et al.*, 2014; Radostits *et al.*, 2007; Uddin *et al.*, 2017).

The prevalence of clinical mastitis is less than 5% in small ruminants, while the subclinical mastitis is much more common with a prevalence that ranges from 18 to 34% (Chingwen *et al.*, 2002). Mastitis in small ruminants is mainly subclinical and results in decreased milk yield, may lead to growth retardation and higher mortality rate among suckling kid and lambs (McDougall *et al.*, 2002). The most common causes of subclinical mastitis in most herds or flocks are bacterial in origin. The bacterial agents isolated from mastitic milk in small ruminants are mainly; *Staphylococcus* species, *Streptococcus* species, *Pasteurella* species, *Pseudomonas* species, *Proteus* species, *Salmonella* species, *Corynebacterium* species, *Bacillus* species and *E. coli* (Contreras *et al.*, 2007). Among the bacterial agents, *Staphylococcus* species have been implicated as the leading causes of subclinical mastitis with higher prevalence in goat and sheep. Especially,

Coagulase-Negative Staphylococcus species (CNS) has been the most common reported cause of sub-clinical mastitis (Bergonier *et al.*, 2003b).

Early diagnosis of subclinical mastitis is vital because changes in udder tissue take place much earlier than they become apparent. Various approaches, based on physical and chemical changes of milk and cultural isolation of organisms (gold standard) are practiced for the diagnosis of subclinical mastitis in small ruminants (Contreras *et al.*, 2007). To diagnose the SCM, indirect tests such as CMT, WST and SCC are commonly used both in cows and small ruminants. White Side Test (WST) is also an indirect, easily applicable screening test for sub clinical mastitis in which 4% sodium hydroxide solution are used as reagent (Contreras *et al.*, 2007).

Characterizing the etiology of bacterial infection is critical for understanding the grounds for establishing of infection and devising appropriate treatments to control the contagion. However, very little research has been done to explore the patterns of intra-mammary infections acquiring in small ruminants (Leitner *et al.*, 2007). Therefore, this study was gird with the objectives of: To estimate the prevalence of subclinical mastitis among small ruminants in Jimma town, Southwestern, Ethiopia; to isolate bacterial pathogens responsible for the occurrence of subclinical mastitis in small ruminants; and to identify risk factors associated with the development of subclinical mastitis in small ruminants.

2. Review of Literature

2.1. Mastitis in Small Ruminants - The term mastitis was derived from the two Greek words "*Mastos*-"(for breast) and "*-itis*" (for inflammation) (Doğruer *et al.*, 2010). It is one of the main udder problems and defined as an inflammation of the mammary glands characterized by changes in the physico-chemical features of milk and pathological changes in the glandular tissue often with non-specific and complex factors responsible for its occurrence (Radostits *et al.*, 2007). In small ruminants, mastitis is a very significant disease that affects not only the meat and milk production, but also the well-being of shoats and human health as well (Abdullah, 2016; Tolone *et al.*, 2016).

2.2. Types of Mastitis in Small Ruminants - Mastitis are a disease that occurs in several different forms. Generally in animal, mastitis is divided into clinical and sub-clinical forms (Doğruer *et al.*, 2010).

2.2.1. Clinical Mastitis - It occurs by having the evidence of both milk and udder changes with cardinal signs of inflammation upon physical examination, which includes swelling, pain, hotness (heat), redness, or necrosis of one or more halve udders and abnormal discharge of milk (presence of flakes or blood), as well as other systemic symptoms such as anorexia, fever or agalactia (Radostits *et al.*, 2007). Usually the consequence of clinical mastitis is toxemia and gangrenous necrosis of the udder (Omaleki *et al.*, 2011). The clinical syndrome can follow variable courses, ranging from hyper-acute to chronic. The occurrence of chronic disease may produce clinical pictures, which become gradually less clinically evident (Bergonier *et al.*, 2003).

2.2.2. Subclinical Mastitis in Small Ruminants - Subclinical mastitis in small ruminants of Africa causes a significant impact on the production and productivity of dairy goat and sheep's due to its association with the absence of clinical signs to be detected by the clinicians or farmers. Lactating ewes/does with subclinical mastitis does not show any clinical symptoms and giving apparently normal milk for long period which only known after indirect tests done and cultured with bacteriological examinations which makes them positive to mastitis (Kiossis *et al.*, 2007). Identification of animal affected with subclinical mastitis is much more difficult than recognition of those with clinical mastitis. Detection of subclinical mastitis may require some additional indirect tests such as with CMT test, SCC and microbiological tests (Plummer and Plummer, 2011).

2.3. Incidence and Etiology of Mastitis in Small Ruminants - Incidence of the clinical mastitis in small ruminant is usually below 5% and can lead to mortality or culling up to 90% of infected animal in the flock. However, in certain herds and flocks having of poor management system it may exceed 30% (Goodridge *et al.*, 2004). Mostly, sporadic cases of clinical mastitis are caused by *Staphylococcus aureus*, coagulase-negative *Staphylococci* (CNS), *Arcanobacterium pyogenes*,

Corynebacterium, *Pasteurella* species, and *Pseudomonas* species. Outbreak of clinical mastitis most frequently involves *Staphylococcus* species, *Streptococcus* species and other opportunistic agents such as *Aspergillus* species, *Pseudomonas* species, *Burkholderia* species, and *Serratia* species (Croft *et al.*, 2000; Plummer and Plummer, 2011).

The most common cause of subclinical mastitis in most herds or flocks is bacterial in origin. Among them, *Coagulase-Negative Staphylococcus* species (CNS) have been implicated as the leading cause of subclinical mastitis with prevalence rates of 71% and 78%, respectively, in goats and sheep. The second most common reported cause of subclinical mastitis is *S. aureus*, with reported prevalence rates of 8% in goats and 4% in sheep (Bergonier *et al.*, 2003b). *Streptococcus* species also have been isolated in cases of subclinical mastitis with prevalence rate ranges between 1.1% and 6.8% of subclinical mastitis cases (Mork *et al.*, 2007; Hall *et al.*, 2007; Ndegwa *et al.*, 2001).

2.4. Epidemiology of Small Ruminants Mastitis - In dairy animals, mastitis causing pathogens are classified depending on their epidemiological behavior. However, such description is not as clear for small ruminants as it is for dairy cattle. During time of milking of the small ruminants, the likely source of Coagulase-Negative *Staphylococci* (CNS) are the skin on the teats along skin around the inner side of legs; thus, many CNS bacteria become persisted and long-term chronic infections (Piessens *et al.*, 2011). CNS may leak out to the milk from the infected udder during milking and then spread via the milking equipment to other animals. Thus, the source of mastitis pathogens in small ruminants should not be assumed based simply on the behavior of these pathogens in dairy cows (Olechnowicz and Jaśkowski, 2014). The main reservoir of the contagious pathogens is the mammary gland. *S. aureus* and *Str.* Species are considered among the contagious pathogens. Consequently, these bacteria are spread from animal to other or between udders of the same animal during the milking process (Contreras *et al.*, 2003). Apparently this refers to that *S. aureus* germ capable of contaminating different surfaces and easily isolated from the skin and mucosa of the healthy animals. The role of environmental sources of *S.*

aureus is less important than its presence in the mammary gland habitat, but may help in the spreading of infection from milking of the infected animal and milking machines or dairy workers can transmit it, but are not the origin of these infections. Generally, bacteria causing mastitis are often classified as “contagious”; if it is believed that the source of infection of milk is infected mammary glands with subclinical mastitis, or “environmental”; if the bacteria are considered to be opportunistic pathogens that normally reside in the environment around the animals (Ruegg, 2011).

2.5. Important Causative Agents of Subclinical Mastitis in Small Ruminants

2.5.1. Bacterial Agents

Gram-positive agents

Staphylococcus species – These species are Gram-positive cocci, non-motile, non-spore forming, aerobic and facultative anaerobic bacteria. They are found in clusters like bunches of grapes and appear violet color when stained, catalase positive and usually oxidase-negative. Also, they could hydrolyse arginine and produce acetone and attack sugar by fermentation (Barrow and Feltham, 2004). Considering the fact that, *S. aureus*, Coagulase-negative Staphylococcus species (CNS) such as *S. epidermidis*, *S. saprophyticus* and *S. intermidus* are the important causative agents of subclinical mastitis in small ruminants (Windria et al., 2016).

Streptococcus species - These species are also Gram-positive cocci in pairs or chains, non-motile, non-spore forming, aerobic and facultative anaerobic organisms. They are both catalase and oxidase negative, and ferment carbohydrate (Barrow and Feltham, 2004). Despite the pathological importance of *Streptococci* species in mastitis, they do not represent a group of especially prevalent pathogens on small ruminant flocks and are usually reported in somewhat less than 5-10% of cases of sheep and goats mastitis. However, they majorly cause clinical mastitis, and a number of epidemiological studies on subclinical mastitis even report the absence of these pathogens. This situation seems clearly different in certain areas of dairy small ruminant production, in which major problems on

farms are diagnosed due to *Str. agalactiae* (Contreras et al., 2003).

Enterococcus species - *Enterococcus species* are Gram-positive cocci in pairs or short chains. They are non-motile (except few species), non-spore forming, aerobic and facultative anaerobic, catalase and oxidase-negative. They ferment carbohydrates (Barrow and Feltham, 2004). Those Gram positive bacteria are associated with mastitis in sheep, goat and cattle; include *Enterococcus faecium* and *Enterococcus faecalis* (Contreras and Rodríguez, 2011).

Gram-negative agents

E. coli - *E. coli* is Gram-negative rods, motile, aerobic and facultative anaerobic. Catalase-positive, Oxidase-negative, ferment sugars and produce gas (Barrow and Feltham, 2004). They are organisms commonly found in the environment in order to cause the coliform diseases and considered as opportunistic pathogen.

Pasteurella species – *Pasteurella species* are Gram-negative rods, non-motile, aerobic and facultative anaerobic. Usually, they are Catalase-positive, Oxidase-positive and can ferment sugar (Barrow and Feltham, 2004). The main source of infection from *P. haemolytica* for the teat of the ewe is the mouth of the lamb. In dairy sheep, where ewes are milked by hand or machine after lambs are removed, the incidence of *P. haemolytica* mastitis is dramatically reduced after lambs are weaned because there is no longer presence of organism on the teat of the ewe.

Mycoplasma species - *Mycoplasma* is a genus of bacteria that lack a cell wall around their cell membrane. They do not possess rigid cell walls, they are resistant to antibiotics such as penicillin, which interfere with the synthesis of bacterial cell walls. *Mycoplasma species* are the smallest bacterial cells yet discovered typically, about 0.1 µm in diameter. It can be present in the milk of sheep and goats and can cause mastitis in the animal (Quinn and Markey, 2003). Contagious agalactia (CA) is a serious disease of small dairy ruminants that has a substantial economic impact on the goat and sheep milk industries. The main etiological agent of the disease is *Mycoplasma agalactiae*, although other species, such as *Mycoplasma mycoides subsppcapri*, *Mycoplasma capricolum* Sub

spp. Capricolum and *Mycoplasma putrefaciens*, are pathogenic in goats (Contreras and Rodríguez, 2011).

2.5.2. Viral Agents

The mammary gland is more susceptible to injury by ovine progressive pneumonia virus (OPPV), nine of 15 experimentally infected sheep and all six naturally infected sheep had lympho-plasmacytic mastitis. *Caprine arthritis-encephalitis virus* (CAEV), like other *Lentiviruses*, causes a persistent lifelong infection. The CAEV induced subclinical infection in most goats. Most of infected does are likely to have a viral infection of the mammary gland, which is a target organ for the virus.

2.5.3. Fungal Agents

Cryptococcus neoformans which is yeast causes mastitis in cattle, buffaloes and experimentally in goats. *Cryptococcus* species inoculation into the glands of 10 goats resulted in mastitis in all of them but, there was no spread to the un-inoculated udder half. Mastitis may be acute with severe swelling of the gland and the supramammary lymphnodes, severe fall in milk yield and appearance of viscid mucoid gray white secretion. It is of public health importance especially where milk is taken without pasteurization since it causes human *Cryptococcoses*.

2.6. Diagnosis of Mastitis in Small Ruminants

2.6.1. Physical Examination

Visual examination and Udder palpation - Clinical examination of the small ruminant udder should be performed in order to palpate possible lesions on the skin of the udder and note visually color changes, injuries and nodules as well as changes in the general shape of the udder for example, increase in size and atrophy. Pain is one of clinical sign of the mastitic udder; therefore, any pain reaction during palpation should be noted. Furthermore the presence of nodules or hardness within the gland should be recorded. Each teat was held between the thumb and the index finger, and palpated throughout its length to evaluate its shape, size and consistency; teats of the same udder were compared with each other (Mavrogianni *et al.*, 2004). Finally, the supra-mammary lymph nodes of the examined animal should be palpated, in order to detect

possible alterations in size or consistency (Mavrogianni *et al.*, 2005).

Visual examination of the mammary gland secretion

- The first few streams of milk are to be drawn from the teat onto a paddle or on the palm of the gloved hand of the investigator. Expression of milk can be evaluated; for example, expression in drops can be indicative of teats enosis. Then, presence of abnormal features in mammary secretion (e.g., clots, flakes, tints) should also be recorded (Mavrogianni *et al.*, 2004).

2.6.2. Chemical Examinations

Milk tests - There are various tests used to determine type of the causative organisms and the infection level of the herd or even individual animal. Wherefore making recommendations about a diagnostic test, it is important to consider the consequences of false-negative and false-positive results. In the case of mastitis, the costs of treatment or culling of positive animals are important factors, and the aim is generally to reduce the overall prevalence of infection without necessarily eliminating the disease; therefore, specificity takes priority. However, to eliminate a disease, the sensitivity would have a higher priority than specificity (Clements *et al.*, 2003; Lafi, 2006; Rahman *et al.*, 2019). Appropriate tests include California Mastitis Test (CMT) and Somatic Cell Count (SCC) are regarded as the best indirect tests to diagnose intramammary infections (IMI) in both sheep and goats, when they are interpreted correctly (McDougall *et al.*, 2001; Bergonier and Berthelot, 2003; Luengo *et al.*, 2004; Persson and Olofsson, 2011).

Californian mastitis test (CMT) - The California mastitis test (CMT) is the quick and easy method used to detect subclinical mastitis. Agents in the reagent react with the DNA present in the epithelial and inflammatory cells to form a gel. The more DNA in the milk, the more gel is formed and the higher a count of the bacteria. The results are essentially subjected to assessment of the amount of gel, the degree of precipitation and gel formations are graded. The grades were: 0) no precipitate; 1) trace precipitate; 2) distinct precipitate/weak gel formation; 3) distinct gel formation; and 4) strong gel formation.

Somatic cell count (SCC) - The most common method for the detection of high bacteria counts in milk is the measurement of the somatic cell count (SCC), which gives an indication of whether an immune response to mastitis has begun in an animal. The numbers of epithelial and inflammatory cells (per ml) are counted in a milk sample, usually taken on the 'test' days at regular intervals during lactation. The numbers of somatic cells indicate the degree to which an immune response has been mounted against organisms that cause intra mammary infections (IMI). The SCC can be regarded as a surrogate measure of mastitis (Barillet *et al.*, 2001). It is important to know the threshold level of SCC because it has impact on the milk production and health of sheep and goats in dairy animals, current estimates in the range of 600,000 to 800,000 cells per ml indicate presence of infection and animal with less than 500,000 cells per ml are considered to be 'healthy' (Barillet *et al.*, 2001).

Modified White side test (WST) - California Mastitis Test and Whiteside Test are recommended as threshold values for subclinical mastitis offering at least 93% accuracy of the diagnostic methods. In the WST, nucleic acids of the leucocytes of milk form a sodium salt with 0.4% NaOH producing a gelatinous mass to which serum solids and fat globules become absorbed to produce the characteristic precipitate of the reaction.

2.6.3. Bacteriological Examination of Milk Sample

Bacteriological examination of milk samples remains the 'gold standard' for etiological diagnosis of the mastitis in small ruminants. Isolation of bacteria in pure culture and sufficient growth from only one milk sample from a ewe/doe with mastitis suffices to demonstrate the etiological role of these organisms in the disease (Contreras *et al.*, 2007). Repeat samplings and bacteriological examinations are required only in following up efficacy of treatment regimes or monitoring control programs. The greatest advantage of bacteriological examination of milk samples is the possibility to demonstrate the etiological role of the isolated organisms in mastitis. Nevertheless, the method requires some time for definitive results and cannot be performed onsite (Fragkou *et al.*, 2014). Specificity and positive predictive value were higher for post-milking samples than for pre-milking samples and false positive diagnoses were more frequent for

pre-milking samples. Post-milking samples should be used to diagnosis of small ruminant intra-mammary infection (Sánchez *et al.*, 2004).

2.7. Factors that Affects Occurrence and Timing of Onset of the Mastitis

Various factors have been associated with the occurrence of mastitis in small ruminants such as parity, breed, udder halves, region and the number of lambs/kids born. Notable suckling two or three lambs is associated with a greater mastitis risk than suckling only one lamb per ewe (Arsenault *et al.*, 2008; Waage and Vatn, 2008). Factors related to the herd such as number of parity, stage of lactation and age, have been demonstrated to significantly influence prevalence of mastitis in goat and sheep; usually, infection rates are less frequent at the first kidding/lambing, and increase with age (McDougall *et al.*, 2002; Moroni *et al.*, 2005b). In addition, production forms and management practices may give rise to differences both in the epidemiology, bacteriology and clinical manifestations of mastitis. The timing of the onset of mastitis is important, in previous review of five studies by Bergonier *et al.*, (2003); they reported that the majority of cases of mastitis occurred from the beginning of machine milking and during the first third of lactation.

2.8. Economic Impacts of Mastitis in Small Ruminants

Mastitis is of importance from three perspectives (Bergonier and Berthelot, 2003): hygienic, legal, and economic, that represent in reduced growth of lambs and their mortality, as well as mortality of ewes, treatment costs, reduced milk production and milk prices dependent on cellular quality in certain areas. The worldwide losses due to the disease are approximately \$35 billion annually (Bilal *et al.*, 2004; Abdullah, 2016). Mastitis has a major impact on both economy and animal welfare in small ruminant production and associated with a reduction of milk yield and quality in ewe due both clinical and subclinical ewe's udder infection (Albenzio *et al.*, 2002). While, mastitis caused by microbial infections in dairy goats reduces milk yield, modifies milk composition, and potentially contributes to morbidity in herds (Gutierrez-Chavez *et al.*, 2016). In this context, manufacture of products from the meat and milk of small ruminants presents an

opportunity to improve the economics of farming worldwide, thus, economic losses due to mastitis are of major concern to the dairy industry. Loss of milk quality caused by subclinical infection in dairy sheep has a negative effect on cheese manufacture (Abdelgawad *et al.*, 2016).

2.9. Treatment of Mastitis in Small Ruminants

Treatment techniques of mastitis in small ruminant had been described by many hypotheses (Bergonier and Berthelot, 2003). Theoretically, the aim of 'in-lactation' treatment is the clinical and bacteriological cure of infected halves of clinically affected ewes with mastitis and functional recovery. In fact, for acute and per-acute mastitis, the aim is to avoid death and allow culling. In any case, affected animal must be removed from the dairy flock until culling or complete recovery. Antimicrobials are used in treatment of mastitis predominantly during lactation and, less frequently, during the dry period (Onni *et al.*, 2011).

2.9.1. Antimicrobial Therapy of Small Ruminant's Mastitis - Bacterial infections are the predominant cause of both clinical and subclinical mastitis and antimicrobial agent remain a component of both mastitis treatment and control. The successful control depends on antibiotic treatments used. The use of antibiotics in food animals is under challenge, particularly use of broad spectrum, multi-component products and use of prophylactic treatment which should be practical and cost effective (Oliver *et al.* 2011). Using antibiotic in the treatment of clinical infections need selection of the most appropriate antibiotic and requires knowing the organism's identity, the organism's susceptibility to a particular antibiotic, the site of the infection (targeted tissue in present study is udder), individual factors of ewe/doe, the safety of the antibiotic in use and the cost of antibiotherapy (Shahen *et al.*, 2019; Richard *et al.*, 2012).

2.9.2. Antibiotherapy of Small Ruminants Mastitis During Lactation Period - The major antibacterial used as treatments for mastitis have been directed against Gram-positive organism are particularly, *Staphylococci* and *Streptococci*. In addition, application of management practices that decrease the prevalence of contagious pathogens such as *Strepto-*

coccus species and *S. aureus* has shifted the focus of mastitis control and economic losses to environmental pathogens that are associated with more frequent episodes of subclinical mastitis. That way, antibiotherapy should start immediately after detection of the first signs of the disease on mastitic udder and should be performed using effective antimicrobial agents (Erskine *et al.*, 2003).

2.9.3. Dry-off Treatment of Mastitis - Indiscriminate use of antimicrobial agents can result in bacterial resistance and super infection, prophylactic use of antibiotics is restricted to clinical situations in which the benefits outweigh the potential risks. The use of antibiotics for the prevention is better than the treatment of infections and the duration of prophylaxis should be closely observed to prevent unnecessary antibiotic exposure (Richard *et al.*, 2012). This prophylaxis program was applied in previous study by Kiossis *et al.*, (2007) to test the effectiveness of a standard treatment for the control of subclinical mastitis during lactating period in a modern, commercial dairy sheep farm, resulted in limitation of subclinical mastitis during lactation and a better health status for udders entering the dry period. While the intra-mammary ampicillin dicloxacillin treatment had the best treatment rates, the combination of intramuscular amoxicillin clavulanic acid was also successful. Intramuscular amoxicillin-clavulanic acid as the sole treatment was not as effective as intra-mammary therapy (Doğruer *et al.*, 2010).

2.10. Control Techniques for Small Ruminants Mastitis

The fight against mastitis should not be limited to treating isolated clinical episodes, but also requires the surveillance and control of both, clinical and sub-clinical mastitis (Paterna *et al.*, 2014). The knowledge of risk factors and etiological agents involved are also important in order to recommend specific and efficient control measures for both clinical and subclinical mastitis (Arsenault *et al.*, 2008). Prevention of mastitis in small ruminants can be described upon programs that include vaccination, culling, application of good husbandry control and proper maintenance of milking machines, better milking routine without over milking and removal of clusters without impacts, and hygiene

after milking. Improved techniques depend on a better understanding of the nature of predisposing factors and breeding for resistance are the approach that is considered as a sustainable methods for mastitis control (Conington *et al.*, 2008).

3. MATERIAL AND METHODS:

3.1. Study Area - The current study was conducted from October, 2017 to April, 2018 in the Southwestern Ethiopia, in and around Jimma town, the capital of the Jimma zone. The town is about 346 KM away from Addis Ababa, capital city of Ethiopia. It has a latitude and longitude of 7°40'N 36°50'E/7.667°N 36.833'E respectively. The altitude of Jimma is 1780 meters above sea level (masl). It has a tropical rain forest climate with bimodal rainfall of the long annual wet season from March to October. The temperature at Jimma is in a comfortable range, with the daily mean staying between 20°C and 25°C year-round. The production system observed around the area was crop-livestock mixed production system (CSA, 2010).

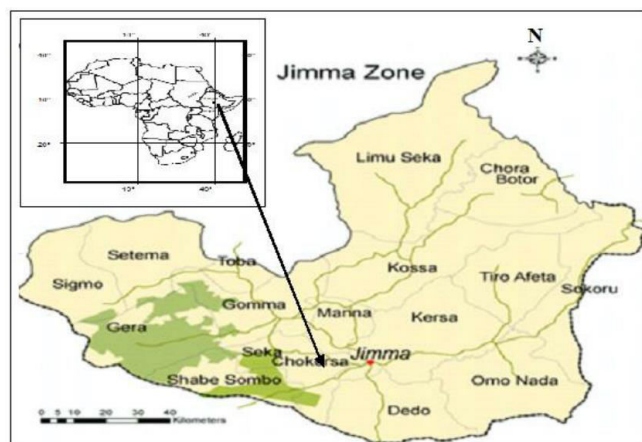
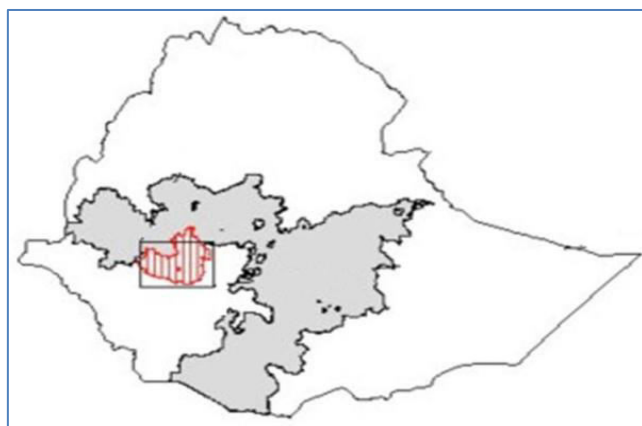


Fig 1: Map of the Jimma zone, Oromia regional state, Southwestern, Ethiopia.

Based on the 2007 census conducted by the Central Statistical Agency of Ethiopia (CSA), Jimma zone has total population of 2,486,155 with an increase of 26.76% over the 1994 census, of which 1,250,527 are men and 1,235,628 women; with an area of 15,568.58 square kilometers. Jimma zone has a population density of 159.69 while 137,668 or 11.31% are urban inhabitants, a further 858 or 0.03% are pastoralists. According to the zone livestock and fisheries agency; Jimma zone has an estimated 1,718,284 heads of cattle, 466,154 sheep, 194,677 goats, 74,774 horses, 40,555 donkeys and 30,541 mules population (CSA, 2007).

3.2. Study Population - Animals included in this study were apparently healthy lactating local breeds and extensively managed small ruminants (sheep and goats) from selected four different districts in and around Jimma town, Jimma zone, Southwest, Ethiopia. A total of 186 small ruminants (75 sheep and 111 goats) from different districts and animals found at different age, stage of lactation, and parity numbers were considered. Small ruminant populations were classified in to three groups of age depending on their parity number which includes; adult groups for one to two parity, medium groups for three to four parity and older groups for five and above numbers of parity by asking the owners of animals.

3.3. Study Design - A cross sectional study was conducted from October, 2017 to April, 2018 in Southwestern Ethiopia, in and around Jimma town to estimate the prevalence of subclinical mastitis in small ruminants at the study area. In the current study, the districts found in Jimma zone were selected and grouped in a cluster, and within selected districts (Tiro Afeta, Kersa, Gumay and Jimma town) certain villages were selected according to simple random for milk samples collection.

3.4. Sample Size Determination - To determine the prevalence of small ruminant subclinical mastitis in and around Jimma town, an expected prevalence of 50% was taken into consideration. The sample size for the current study was determined by the epidemiological formula with 95% confidence interval and 5% absolute precision:

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

Where: n = required sample size, P_{exp} = expected prevalence, $1.96^2 = 95\%$ confidence interval d^2 = desired absolute precision. Based on the formula, the minimum sample size required was 384 from 192 target animals. However, in present study, since it is a dry season of the year from December to February months at the study area, it was impossible to collect 384 samples from the target population because of weaning of lambs and kids in the season the study was conducted. Therefore, maximum effort was exerted to get samples during the study period and a total of 372 milk samples were collected from 186 small ruminants.

3.5. Sampling Procedures/Methods - In the current study, apparently healthy lactating small ruminants were sampled to find the target disease by excluding the animals with physically injured udders. From a total of 186 apparently healthy lactating sheep and goats sampled in the study area, 372 milk samples were collected and tested with CMT reagent. During the time of sample collection from the animals, all necessary hygienic protocols were maintained in order to avoid false-positive and false-negative results.

3.5.1. Milk Sample Collection Procedures - Milk samples were collected according to the National Mastitis Council (NMC). After the udder halves have been washed with tap water, soap and dried with clean dry cloth, the teat ends were swabbed with cotton soaked in 70% ethyl alcohol for purpose of disinfection. Then after, teat ends dried, the first three milking streams were discarded and approximately 10ml of milk was collected aseptically from each halves in to sterile test tube. Each sample was labeled legibly and accompanied by necessary identification information which includes date of sampling, species of the animals, type of sample, halves of udder, age of the animal, parity number, stages of lactation, and area from which the samples were taken.

3.5.2. Californian Mastitis Test (CMT) of Milk Samples - Milk samples taken from each udder halves were examined by CMT test and this screening test was conducted according to the standard procedure (Quinn et. al., 2002). In current study, all samples were examined on the field by using CMT reagent and CMT negative samples were decided by absence of gel form-

ation and taking grade 0 and trace; then, they were recorded and discarded. However, CMT positive milk samples were whose moderately and highly form precipitation during mixed with CMT reagent and transported at +4°C by packing within the icebox to laboratory for bacteriological tests and preservation purpose until procedures were completed according to the standard protocols with NMC.

3.6. Laboratory Procedures - In present study, laboratory procedures used were bacteriological tests. A loop full of CMT positive milk sample was streaked on blood agar base (Oxoid, UK) enriched with 5% defibrinated sheep blood and nutrient agar, and then sub-cultured on selective media for different bacteria using the quadrant streaking method; Mannitol Salt Agar (MSA) for *Staphylococcus* species, Eosin Methylene Blue (EMB) Agar for *E. coli* and Edwards medium for *Streptococcus* species. In addition, the samples were also cultured on MacConkey Agar (MA) for differentiation. All the plates were incubated aerobically at 37°C for 24-48 hours. Then, the plates were examined for gross colony morphology, pigmentation and hemolytic characteristics at 24-48 hrs. For further identification of the organisms, different biochemical tests (Gram stain, Catalase test, Coagulase test, Indole test, Motility test and O-F tests) were conducted (Quinn et. al., 2002).

3.7. Methods of Data Management and Analysis - The collected data was checked, coded and entered in to Microsoft Excel Work Sheet and analyzed by using SPSS 20 software. Descriptive statistics like frequency and percentage were also calculated to describe small ruminant subclinical mastitis prevalence and comparison of the degree of association between risk factors was done by using chi-square (χ^2) test. In all the cases, 95% confidence interval and 5% absolute precision errors were considered.

A P-value < 0.05 was used to verify statistically significance difference and when the P-value was found to be ≥ 0.05 , the difference in prevalence was considered insignificant. The prevalence of subclinical mastitis was calculated according to both the number of the collected samples and total number of the sampled animals described separately.

4. RESULTS:

4.1. Prevalence of Small Ruminant Subclinical Mastitis and its Association With Risk Factors -

From a total of 186 apparently healthy lactating sheep and goats considered, 70(37.6%) of them were found to be infected with subclinical mastitis based on CMT. And out of a total of 372 milk samples collected, 92 (24.8%) of the samples from 70 small ruminants were positive for CMT and bacteriological tests. During the study period, potential risk factors such as species of animals, age, parity numbers and lactation stage

were taken in to consideration to estimate the association with the occurrence of the subclinical mastitis in small ruminants.

4.1.1. Occurrence of Subclinical Mastitis between Species of Animals - Among 75 and 111 sheep and goats species respectively tested with CMT and bacteriological tests, some of them tested positive and others negative with (P=0.107). Observed results indicated that, species as a risk factor is statistically not significant with occurrence of subclinical mastitis in small ruminant populations at study area with a total prevalence of 37.6% (Table 1).

Table 1: Overall prevalence of small ruminant subclinical mastitis between species of the animals

Species	No of +Ve	No of -Ve	Total	% of +Ve	% of -Ve	P-Value
Sheep	23	52	75	12.4	28	
Goat	47	64	111	25.3	34.4	
Total	70	116	186	37.7	62.4	0.107

Note: No of +Ve=Number of positive, No of -Ve=Number of negative, % of +Ve=percentage of positive, % of -Ve=percentage of negative.

Table 2: Subclinical mastitis between age categories and among parity numbers of small ruminants

Risk factors	No of animal examined	No of +Ve animals	No of -Ve animals	Percentage		P-Value
				+Ve	-Ve	
AGE						
Adult	49	9	40	4.8	21.5	
Medium	61	15	46	8.1	24.7	
Old	76	46	30	24.7	16.2	
Total	186	70	116	37.6	62.4	0.000
Parity						
1-2	49	9	40	4.8	21.5	
3-4	61	15	46	8.1	24.7	
≥ 5	76	46	30	24.7	16.2	
Total	186	70	116	37.6	62.4	0.000

Note: No of +Ve = number of positive, No of -Ve = number of negative, % of +Ve = percentage of positive, % of -Ve = percentage of negative.

4.1.2. Occurrence of the Subclinical Mastitis among Different Age Categories of Animals - In the study, the difference in age among small ruminants was found to be greatly associated with the occurrence of subclinical mastitis in small ruminants (P=0.00). Out of the 186 animals, 49 (26.3%) were adult and 9 (4.8%) of them were showed positive result for subclinical mastitis of the small ruminant at study area. While among sampled population, 61 (32.8%) were medium and 15 (8.1%) animal numbers showed positive result

for SCM of sheep and goats. Also, from 186 sample small ruminants, 76 (40.9%) were in older group and out of them 46 (24.7%) were found positive for subclinical mastitis.

4.1.3. Occurrence of Small Ruminant Subclinical Mastitis among Parity Numbers - In present study, parity number of the target population were grouped according to their number of lambs/kids gave by asking the owners. Animals were classified in to 3

classes of groups like age and described. Parity number of animals was found to influence prevalence of subclinical mastitis in statistically significant state ($P = 0.00$). For this study, parity numbers and ages of animal were more related to each other based on information gathered from animal owners about number of lambs/kids they gave to them and decision to the age of ewe/doe was made based on their parity numbers (Table 2).

4.1.4. Occurrence of Small Ruminant Subclinical Mastitis among the Stages of Lactation - Out of 186 healthy lactating does and ewes, 63 (33.9%), 69 (37.1%) and 54 (29%) at their early, mid and late lactation stages respectively and showed differences in subclinical mastitis (Fig 1). The prevalence of subclinical mastitis among lactation stages of small ruminants was different but was not statistically significant ($P = 0.09$).

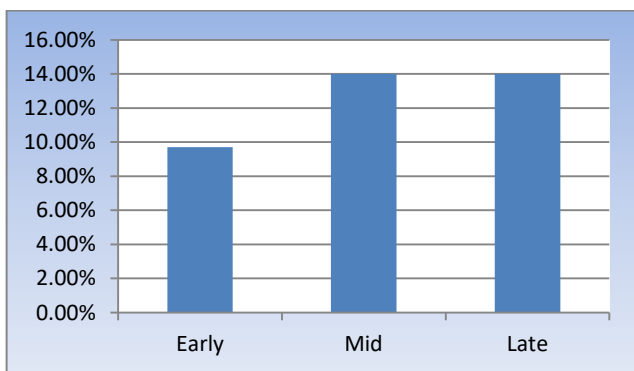


Fig 2: Graphical presentation of SCM prevalence in small ruminants among lactation stages.

4.1.5. Prevalence of Small Ruminant Subclinical Mastitis based on the CMT Test - In current study, 186 healthy lactating sheep and goats were subjected to CMT, out of which 70 (37.6%) animals were positive for CMT, based on degree of gel formation. From 186 animals, 372 milk samples were collected and examined, out of which 92 (24.8%) were positive with CMT (Fig 2). However, these 92 samples were taken from right halve positive and left halve negative or vice versa and/or both halves positive of the 70 sheep and goats. Due to this reason, the overall prevalence calculation was used rather than individual udder halves' prevalence calculation. And the overall prevalence of the subclinical mastitis based on CMT result was estimated to be 37.6%.

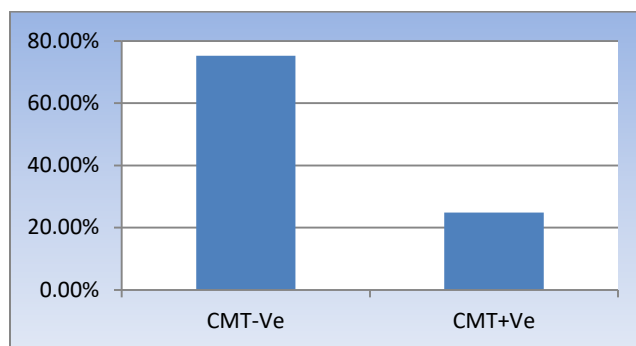


Fig 3: Graphical presentation of prevalence of SCM in small ruminants based on CMT result.

4.1.6. Prevalence of Gram Positive Bacteria in Small Ruminant Subclinical Mastitis - All of the 70(37.6%) CMT positive samples were cultured at bacteriology laboratory on different media, out of which 47(67.1%) samples were positive for Gram positive bacteria based on their Gram stain result.

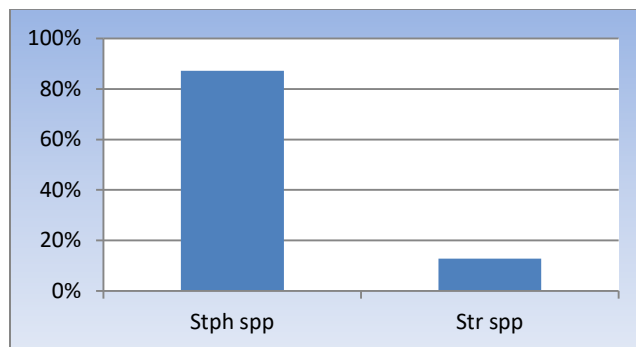


Fig 4: Graphical presentation of the percentage of *Staphylococcus* species and *Streptococcus* species in Gram positive bacterial agents from CMT positive samples (Where: Stphspp = *Staphylococcus* species, Str spp = *Streptococcus* species).

In consideration of individual bacterial isolates, from 41(87.2%) *Staphylococcus* species 15(36.6%), 19(46.4%), 3(7.2%) and 4(9.8%) were *S. aureus*, *S. epidermidis*, *S. hyicus* and *S. intermidus* respectively. And among 6(12.8%) of *Streptococcus* species, 6(100%) of them were *S. agalactiae*, isolated based on the basic standard procedure of bacteriology laboratory.

In the present study, the total population prevalence of SCM caused by *Staphylococcus* species was 22% and the prevalence of *Streptococcus* species was 3.2%. However, the overall prevalence of SCM in small ruminant caused by Gram positive bacteria in study area was 25.2%.

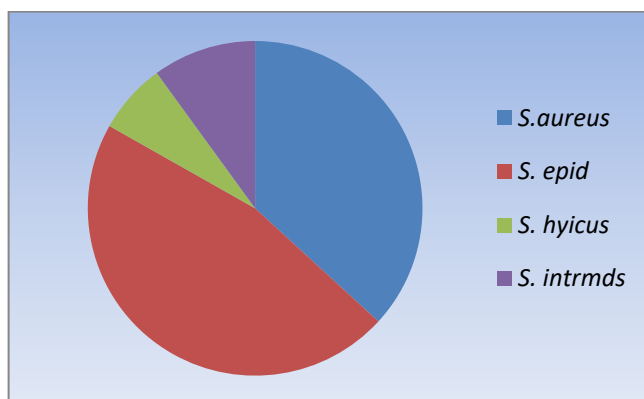


Fig 5: Graphical presentation of the prevalence of isolated *Staphylococcus* species in CMT positive samples from target population.

4.1.7. Prevalence of Gram Negative Bacteria in Small Ruminant Subclinical Mastitis - Out of the 70(37.6%) CMT positive samples cultured at bacteriology laboratory, 23(32.9%) of them had taken Gram negative staining characteristics and the isolated species of bacteria was *E. coli*, with a prevalence of 12.4%, as all stained samples were positive by further biochemical tests done.

5. DISCUSSION:

The overall 37.6% prevalence recorded of subclinical mastitis among small ruminants in the study area was found to closely agree with prevalence reported by Chingwen *et al.*, (2002), who reported the prevalence of SCM in sheep and goats to be between 18% and 34%. Out of 75 lactating ewes, 23 (30.6%) of them shown positive result for the subclinical mastitis. The total prevalence of target disease in small ruminant population at study area was recorded as 12.4%. This infection prevalence is relatively lower when compared with reports in other countries like Jordan (24.8%) by Almajali *et al.*, (2003), Iran (39%) by Batavani *et al.*, (2003), and Portugal (70%-92%) by Quiroga *et al.*, (2003). The prevalence difference observed might be due to geographical variation, breed differences, management system they follow and there was no machine milking system in present study. For this reason, present finding is not agreed with above reports. However, the current result was more related with finding of the Gebrewahid *et al.*, (2012), who reported the prevalence of SCM in sheep as (10.6%) in Kefeta Humera and Tanqua Abergelle districts in Tigray regional state, North Ethiopia. In

order to found result, present report is closely agreed with mentioned above finding in Ethiopia based on similar management system and procedures done to examine the target populations.

In case of goat species, 111 (59.7%) were examined and 47 (42.3%) does were found to be positive for SCM among sampled goats and overall small ruminants population prevalence of SCM recorded was 25.3% at study area. The result demonstrated in this study was greatly lower than the findings reported by Adwan *et al.*, (2005) 52% in Palestine, Swai *et al.*, (2008) 51.1% in Tanzania, and 56% in Nigeria. However, the prevalence recorded in current study was moderately closed to different reports with overall prevalence of 27.3% and 28.7% in Vermont (USA) (McDougall *et al.*, 2002) and in Kenya respectively. But, overall recorded prevalence of the present study in goat was slightly higher than those reported in Ethiopia 18.0% (Gebrewahid *et al.*, 2012) and in South Ethiopia 15.5% (Megersa *et al.*, 2009). All mentioned above differences might be due to many reasons such as; variation in number of sampled animals, difference in host breed and management system risk factors and in present study, there was no machine milking of target populations which might expose the animals to intra-mammary infection.

In present study, age of the animals is considered as one of the important forceful risk factors and clearly significant difference ($P=0.00$) was found between the three groups animal age which were grouped according to their parity number. The highest prevalence of SCM infections were recorded in does and ewes found at older age with prevalence of 24.7%. While, medium class of age was infected at second level with rate of 8.1% and lastly, adult age groups were affected at lower level of SCM with population prevalence of 4.8%.

In this study, the total target animal prevalence of SCM at study area was 37.6%. Present result show that, it is in negotiation with previous reports by McDougall *et al.*, (2002) and Moroni *et al.*, (2005b) which sate that, usually the prevalence of mastitis are less frequent at the first kidding/lambing and increased with age at parallel; i.e higher risk of mastitis were reported in older age of group.

In current study, stages of lactation were also considered as one of the important risk factors and three lactation stages were used, i.e early, mid and late lactation stage. Though, animals at the early lactation stage were less infected with total population prevalence of 9.7% and both animals at mid and late lactation stages were found at equal prevalence of infection in target population of 14%. Present report showed that, the rate of SCM occurrence in small ruminants was increased with prolonged lactation. This result was found to be in agreement with previous reports of Leungo *et al.*, (2004) and Moroni *et al.*, (2004). However, there was no significant statistical association between development of SCM and stages of lactation ($P=0.09$) at the study area. In present report, based on the CMT test done on field at animal side, the prevalence of SCM in sampled animals was 24.8% as the 92 number of samples collected were positive from 70 sheep and goats out of the 186 total populations. In current study, the overall small ruminant subclinical mastitis according to CMT test at target area was recorded as 37.6%. This result is higher when compared with report discussed by Haftay *et al.*, (2014) in Aba'lla district, Afar region the prevalence of SCM reported was 20.6%. This difference might be due to environmental variation, methodologies used and breed differences.

The prevalence of subclinical mastitis caused by Gram positive bacteria in 186 sampled animals at target area was 67.1%. At genus level, 41 (87.2%) and 6 (12.8%) *Staphylococcus* species and *Streptococcus agalactiae* were identified respectively. Among *Staphylococcus* species, there was higher prevalence of Coagulase-negative Staphylococci (CNS) i.e *Staphylococcus epidermidis* at rate of 46.3% followed by *Staphylococcus aureus* at prevalence of 36.6%, *Staphylococcus intermidus* at rate of 9.8% and finally *Staphylococcus hyicus* at 7.3% were isolated from CMT positive samples. The total population prevalence of SCM at study area caused by *S. epidermidis*, *S. aureus*, *S. intermidus* and *S. hyicus* were 10.2%, 8.1%, 2.2% and 1.6% respectively. The present result was found more related and in agreement with previously reported findings in Spain. However, the prevalence of *S. aureus* in present result is far lower than the report of Winter, (2009) for years 1998 to

2003, the prevalence of *S. aureus* were reported between 20.8% and 46.6% as pathogen of the small ruminant subclinical mastitis. This gap might be due to environmental variation, difference in the management system of the flocks, methodologies used, breed variation, number of animals tested and there was no machine milking in present study. Due to this reason, current result is not in agreed with report of Winter, (2009).

The other Gram positive bacteria isolated in current study was *Streptococcus agalactiae* with the prevalence in Gram positive organism of 12.8% and overall population prevalence of SCM at target area by *Str. agalactiae* was 3.2%. Present result is found in agreement with previous reports of Mork *et al.*, (2007), Leitner *et al.*, (2007), Hall *et al.*, (2004) and Ndegwa *et al.*, (2001). In current study, isolated Gram negative bacteria causing SCM in small ruminant at study area was *E. coli* at total population prevalence of 12.4%. Despite of this, certain studies have reported that Gram negative bacteria rarely induce SCM in sheep and goats and present result is found less related with Gebrewahid *et al.*, (2012) report on *E. coli* as its prevalence was 17.0% reported in Tigray regional state, North Ethiopia. Due to this reason, both results are less agreed. However, the current result is higher than Mbindyo *et al.*, (2014) report of the *E. coli* prevalence in Kenya was 3%. But, present study is lower when compared with report of Iqbal *et al.*, (2004) as he was reported the prevalence of *E. coli* in small ruminant 25%. This difference might be due to geographical variation, tests used in the methodology, exposure rate of hosts to the bacteria, management system of the flocks and there was no machine milking in present study.

6. CONCLUSION AND RECOMMENDATIONS:

The current study estimated a high prevalence of small ruminant subclinical mastitis, occurred due to some bacterial pathogens such as *S. aureus*, *S. epidermidis*, *S. intermidus*, *S. hyicus*, *Str. agalactiae* and *E. coli* with considerable prevalence. The highest prevalence of subclinical mastitis in target population was caused by *Staphylococcus species* followed by *E. coli* and the lowest prevalence was estimated in *Streptococcus agalactiae*. The high prevalence of small ruminant

mastitis in the study area may be associated with the lack of community awareness about the disease and the less attention given by professionals. Due to this reason, reported prevalence of small ruminant sub-clinical mastitis will be very important with regards to animal welfare, economic loss, and public health aspects. Among the risk factors considered (i.e. species, age, stage of lactation and parity numbers) in target population, age and parity number of the animals were indicated to influence the prevalence of subclinical mastitis in statistically significant condition. Based on the above conclusion, the following recommendations are forwarded:-

- Since milk was observed to be consumed only by elders and sometimes by diseased children; community awareness should be performed about the risks associated with consumption of milk of small ruminants, and how to maintain proper udder hygiene before and after milking.
- Subclinical mastitis can cause growth retardation in small ruminants which may lead to poor meat and carcass quality, hide and skin, and wool production; and thus, sheep and goats should be given attention and proper diagnoses and treatment practices be practiced.
- As the older age groups and higher parity numbers are mostly affected classes, owners should remove such animals on time from their flocks, so as to minimize the risks of pathogen distribution.
- Further research should be conducted with emphasis given to the epidemiology of the disease and molecular characterization of infectious pathogens responsible for its occurrence.

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8. CONFLICTS OF INTEREST:

The Authors declare there is no competing interest to publish the present research study.

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