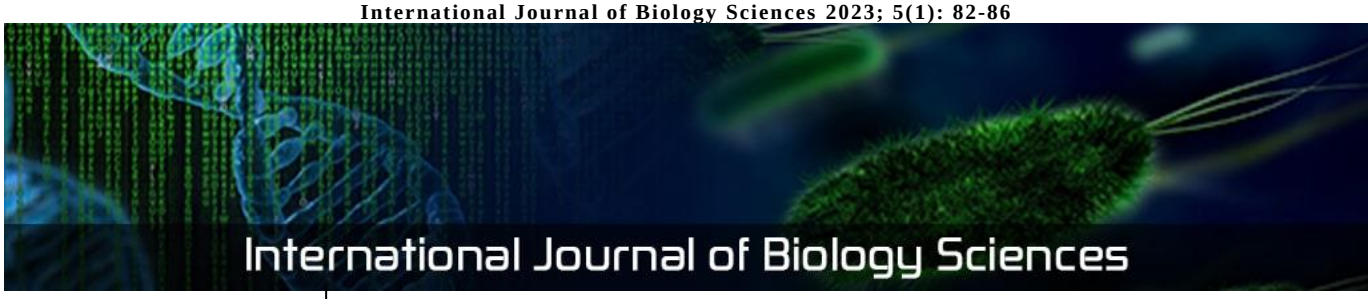

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Ephrem Shimelis
Department of Veterinary Microbiology, Immunology and Public Health, College of Veterinary Medicine, Addis Ababa University, Ethiopia

Corresponding Author:
Ephrem Shimelis
Department of Veterinary Microbiology, Immunology and Public Health, College of Veterinary Medicine, Addis Ababa University, Ethiopia

Prevalence of mastitis causing *Streptococcus* in selected farms in Bishoftu Town, East Shewa, Central Ethiopia

Ephrem Shimelis

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Abstract
Streptococci is among mastitis causing bacterial pathogens in dairy farming in Ethiopia. Accordingly, the cross-sectional study to isolate *Streptococcus* from two selected intensive dairy farms and mastitis cases from Veterinary Teaching Hospital clinic in Addis Ababa University, college of Veterinary Medicine; from July to September, 2021 in Bishoftu town, central Ethiopia. A total of 52 milk samples were collected from the individual lactating cows through randomly sampling from the farms apparently health and purposively from clinical case from veterinary teaching hospital. The milk samples from both clinically infected and apparently health lactating cows and conducting culturing characterization (colony morphology and hemolysis), Gram staining (shape, arrangement and color) and Biochemical tests (catalase and oxidase) to differentiate *Streptococcus* from *Staphylococcus* as *Streptococcus* is catalase negative while *Staphylococcus* is catalase positive. The overall prevalence of streptococci isolated from both milks collected from the clinical and subclinical cow was 18/52 (34.6%) in which the prevalence of *Streptococcus* in milks collected from clinical case and apparently health milk collected from farm were 41.7% and 32.5% respectively with no significant differences (P-value = 0.3). Also the prevalence of *Streptococcus* were 33.3%, 45.5% and 30% at early, mid and late lactating period respectively with significant differences (P-value = 0.05). This indicated that those cows at mid period were highly infected with the *Streptococcus*. Therefore, good farm managements, hygiene of animal's house, maintenance of temperature and ventilation and good milking practice, such as teat disinfection and drying, as well as regular cleaning and checking of the milking machine should be recommended for all farms.

Keywords: Clinical, prevalence, *Streptococcus*, subclinical

1. Introduction
Ethiopia has the largest livestock population of African country with estimated 7.2 million dairy cows that represents a major national resource and form an integral part of the agricultural production system, however, the infectious disease including bovine mastitis is the main economic obstacle disease of dairy cattle.in developing countries, including Ethiopia [10, 22]. It is categorized as the second infectious disease loss directly milk production with higher occurrence frequency the farmer to culling of dairy cows [20]. Bovine mastitis, a complex multi-factorial disease resulted from pathogenic agents invade the udder, multiply, often produce toxins that have a significant detrimental effect on the mammary tissue itself and the general health status of the animal [1]. Over 130 pathogens are known to be associated with bovine mastitis, some of them belonging to the *Streptococcus* genus [13]. Streptococci are among the major mastitis pathogens which have a considerable impact on cow health, milk quality and productivity [18]. *Streptococcus uberis* is one of the most common mastitis pathogens found in dairy herds throughout the world and responsible for a significant proportion of clinical and subclinical mammary gland infections [11]. The disease in dairy herd is of great economic importance due to a reduction in the milk yield, the change in milk quality, the possibility of permanent change to one or more quarter, or even to the entire udder and death of the cow as well as loss due to increased premature culling rate and cost of treatment [17].
Streptococci are Gram-positive bacteria of spherical shape (0.5–2 µm) that usually form pairs or chains.

They are classified on the basis of colony morphology, hemolysis, and serologic specificity into the Lancefield group taxonomic system [13]. *S. agalactiae*, *S. dysgalactiae* and *S. uberis* is usualy associated with bovine [13]. *Streptococcus* uteri's commonly isolated from the dairy farms with clinical and subclinical mastitis infections [15]. Acquaintance through environmental streptococci arises during and between milking, during the dry period or prior parturition of heifers [13]. Chronically affected animals could contribute particularly to the transmission of the bacterium during milking [15]. It can persist in environment up to months particularly under moist cool conditions. It can transmitted by direct contact, fomites or ingestion of not well cocked meat or unpasteurized cow milk of infected cows. These organisms classically inhabit as commensals and can cause opportunistic infections in those hosts. It can be found occasionally in humans [21]. Group B Streptococci is a bovine pathogen causing inframammary infections. The likelihood of GBS interspecies transmission is largely unknown. On-farm interspecies transmission of GBS between people and cattle is possible and a strain capable of colonizing different hosts and environments, including the human throat and rectum, the bovine udder and rectum, and the farm environment. Other strains, which are currently mainly found in cattle, such as members of CC61/67 and CC103, could emerge from the bovine reservoir as a commensal or pathogen of humans [5].

Mastitis is an important in Ethiopia where no routine prevention and control practices are in place [2]. In Ethiopia, subclinical mastitis is considered to be an important challenge for the dairy development [5]. Mastitis has been contributing to reduced milk production and a major source of economic loss to the dairy industry, through reduced milk yield and quality, cost of drugs and veterinary treatment, discarded milk, and forced culling estimated the economic losses from urban and peri urban areas of Addis Ababa, to be US$58 and 78.65 per cow and per lactation, respectively. In addition to its economic impact, *Streptococcus* agalactiae; group B *Streptococcus* (GBS), is the major etiologic agent of invasive neonatal infections in humans in industrialized countries, causing sepsis, pneumonia, meningitis, Osteomyelitis, and soft tissue infections [16]. In Ethiopia, a few studies have been conducted with the purpose of estimating the occurrence of bovine mastitis due to *Streptococcus.* So that information about the prevalence of the *Streptococcus* and factors associated with the disease as well as the pathogen involved is essential in designing prevention and control measures against the disease. Therefore, the current study was conducted to estimate the prevalence, to assess the risk factors and to isolate the *Streptococcus* from the dairy cow milk in the study area through the Morphological characterization of the colony on Blood agar culture, Gram's staining and Biochemical characterization of the isolates.

2. Materials and Methods

2.1 Study design, Sample size determination and study Population

A cross-sectional study was conducted from July to September 2021. Milk samples were collected from the individual lactating cows through randomly sampling from the farms and purposively from clinical case from veterinary teaching hospital. Sample size was determined based on time availability and accessibility of dairy farms. Totally, 53 samples were collected from apparently lactating cows from CVMA farm and Ethiopian Meat and dairy industry development institute farm randomly and clinical case with mastitis's sign from Veterinary teaching hospital in CVMA purposively. Accordingly, 20 milk samples from Ethiopian meat and milk institute, 20 samples from CVMA farm and 13 clinical cases from CVMA teaching hospital. Specifically, the study was target to isolate and identifying the streptococci that could cause the mastitis in lactating cows by taking the milk samples from both clinically infected and apparently health lactating cows. All milk samples were collected from cows with clinical mastitis together with samples without clinical signs. Milk samples were collected promptly into sterile tubes, and then the samples were stored on ice and immediately transported to our laboratory for analysis within 6 hours of sampling.

2.2 Study methodology

2.2.1 Clinical examination of the udder

The udders were examined visually and then palpated to detect any possible fibrosis, inflammatory swelling, and atrophy of the tissue by a veterinary practitioner. The size and consistency of the mammary quarter were 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 were inspected visually for the presence of any flakes, clots, pus, watery appearance, blood and color change.

2.2.2 Bacterial isolation and identification

Streptococcus was isolated by conducting culturing characterization (colony morphology and hemolysis), Gram's staining (shape, arrangement and color) and Biochemical tests (catalase and oxidase) to differentiate *Streptococcus* from *Staphylococcus* as *Streptococcus* is catalase negative while *Staphylococcus* is catalase positive. 10 µl was streaked onto 5% sheep blood agar (Blood agar plate, Creative Life Science, Taiwan) and incubated aerobically at 35–37 °C for 18–24 hours. After incubation, bacteria were recognized as streptococci based on colony morphology and Gram's staining [7]. Firstly, identification of the bacteria was performed by appreciating the growth on blood agar plate. Then followed by colony morphology gross examinations (colony size, shape, and color), hemolysis (presence or absence and type of hemolysis), gram stain staining (Gram-positive or negative, bacterial shape, structure, arrangement), then Gram-positive culture on nutrient agar which then were incubated aerobically at 37 °C for 24 to 48 hours for growth of bacteria. Identification of the bacteria on primary culture was done on the basis of colony morphology, hemolytic characteristics, and Gram's stain reaction including shape and arrangements of the bacteria. The small-medium sized colonies that were hemolytic or non-hemolytic on 5% sheep blood agar and yielding gram positive cocci were sub-cultured onto nutrient agar to obtain a pure isolate for further identification and subjected to catalase and oxidase tests.

Fig 1: Hemolysis Effect of *Streptococcus* on Sheep Blood Agar

2.3 Data analysis

The raw data generated from the study were arranged, organized, coded and entered to Excel spread sheet (Microsoft office excel 2010). Then the data was analyzed through descriptive analysis with percentage. The results of analyses were mostly described in proportion. Proportion was estimated as the numbers of samples detected positive to *Streptococcus* from the total sample tested.

3. Results and Discussion

3.1 Prevalence of Streptococci species

Streptococci are reported to be among the main pathogens causing bovine mastitis all over the world [13]. Streptococci are regularly isolated from mastitis infected in dairy cows with only incomplete information about the antimicrobial susceptibility of these organisms [14]. The most common risk factors associated with mastitis caused by *Streptococcus* is milking hygiene and practical management [7]. Isolation and identification of *Streptococcus* causing mastitis is important for a rapid treatment, control and prevention of bovine mastitis circulating in dairy farms by giving information on the prevalence of the *Streptococcus* to decision makers and stakeholders (Kabelitz *et al*., 2021) [31]. Therefore, the aim of the current study was to determine the prevalence of *Streptococcus* causing mastitis in Bishoftu Town, central Ethiopia.

The overall prevalence of streptococci isolated from both milks collected from the clinical and subclinical cow was 18/52 (34.6%) (Table 1). The prevalence of streptococci obtained in the current study was higher than that reported by Fesseha *et al*., (2022) [10] who identified streptococci as a causes of bovine mastitis in central Ethiopia with the prevalence of *Streptococcus* species (24.3%) and Abebe *et al*., (2020) [2] who identified 18.6% *Streptococcus* spp as risk factor for mastitis in his study conducted on the Prevalence, risk factors and bacterial causes of bovine mastitis in southern Ethiopia; but the prevalence of *Streptococcus* spp obtained in the current study was lower than that obtained by Abd *et al*., (2020) [1] who reported *Streptococcus dysagalactiae* (23%) and *Streptococcus agalactiae* (20.1%) in his study of "Antimicrobial profile of multidrug-resistant *Streptococcus* spp. isolated from dairy cows with clinical mastitis.

Table 1: Isolation and identification of *Streptococcus*

Risk factors		No. Observed	Positive	Prevalence	Chi-sq	P-value
Lactation period	Early	21	7	33.3%	4.25	0.05
	Middle	11	5	45.5%		
	Late	20	6	30%		
Age	Adult	23	7	30.4%	17	0.03
	Young	29	11	37.9%		
Farms	CVMA	20	10	50%	3.8	0.06
	EDIDI	20	3	15%		
Health status	Clinical	12	5	41.7%	1.75	0.3
	Sub-clinical	40	13	32.5%		
Total		52	18	34.6%		

In the current study the prevalence of streptococci isolated from the milk collected from clinical cases from Veterinary teaching hospital and apparently health cows in college of veterinary medicine farm and Ethiopian dairy industry development institute farm were 41.7% and 32.5% respectively with no significant differences (P-value = 0.3) between clinical and subclinical cases from which samples were collected. These result indicated the great differences with the report of Tora *et al*., (2022) [20] who reported the prevalence of Streptococci species as risk factor of Bovine mastitis with the prevalence of 10.6% and 8.1% for clinical and subclinical mastitis, respectively according to his report of the Bacterial profile of bovine mastitis in Ethiopia: A systematic review and meta-analysis. The prevalence of *Streptococcus* between the farm and clinical case at VTH and also among the farms were different in which CVMA farm (20%), followed by Ethiopian meat and milk institute (15%) and lower prevalence in clinical case at VTH (7.69%). The differences of environment between dairy farms, for instance, temperature, humidity, and even the design of barns, results in the difference of the dominant agents that cause mastitis. Moreover, several previous studies have determined numerous risk factors of streptococcal mastitis, such as high yields, long days in milk, multiparous cows, concave teat ends, poor sanitation, liner slips during milking and the use of straw or manure solids as bedding materials [7]. The effect of poor sanitation and improper hygienic measures inside the two dairy farms under study was clear in the current results of microbial isolation from the examined milk samples as high incidence rate of udder co-infections and high incidence rate of environmental microbes. Furthermost of the farm hygienic management of the milking areas, milk containers sanitation, udder and teats washing, separate use of towel for each dairy cow and milker hygiene were not fully applied by most of the farm owners [13].

When comparing the number of samples expected to be positive by the lactating period the prevalence was 33.3%, 45.5% and 30% at early, mid and late lactating period respectively with significant differences (P-value = 0.05) (Table 1). This indicated that those cows at mid period were highly infected with the *Streptococcus*. Also, when the result obtained are compared by the age of the cow the obtained results indicate the prevalence of 29.7% and 37.93% for young and adult respectively. Higher incidence at early lactation period than late in the present study is in agreement with the findings of Abureema *et al*., (2014) [3] who indicated *S. uberis* to be the most common isolate at early lactation. However, Chairman *et al*. (2012) [6] noted *S. uberis* to be dominant pathogen at all stages of lactation since *S. uberis* mastitis is mainly the result of heavy contamination of the teats and udder with water, mud and faecal matter at any stage during lactation [19]. however, the prevalence of lactating periods at middle and late period were different,; the prevalence at early period was agreed with the report of Lakew *et al*., (2019) [16] who found the early, middle and late (35%, 13.02% and 14.84% respectively) with the isolation and identification of *Streptococcus agalactiae* from farms in and around Haramaya district, eastern Ethiopia.

The prevalence of streptococci in young is lower than that of adult cow. So that the result in current study was agreed with the review of Kromker *et al*., (2014) [15] who stated that *S. uberis* infections are exhibited as acute mastitis, usually during the later lactation and showed that in older cows, prevalence increased with the lactation stage, while during early lactation, there were no significant differences between primiparous and multiparous cows. (Kromker *et al*., 2014) [33]. Contagious bacteria have the potential to spread within a herd easily and widely. In contrast, environmental pathogens are able to survive outside the host and are part of the normal microflora of the cow's vicinity. Exposure through environmental streptococci occurs during and between milking, during the dry period or prior parturition of heifers. The pathogen exposure is related to their environmental abundance, which is influenced, e.g., by humidity and temperature. Environmental pathogens invade the udder when the teat channel is opened after milking or after damage (Kabelitz *et al*., 2021) [31]. (Eg & Km, 2021) [9] revealed that mastitis is the major problem in smallholder dairy farms specially the sub clinical form, so that creation of awareness about the importance and prevention of subclinical mastitis among smallholder dairy farmers, milking infected animals and their respective quarters at last and periodic monitoring of infection status of the udder is recommended (Eg & Km, 2021) [9]. Subclinical mastitis is a complex disease and the prevalence could be affected due to variation in management system, age, milk yield, body condition, parity, environment and other conditions. Thus, these factors might have contributed to the observed differences in prevalence of subclinical mastitis (Birhanu *et al*., 2017) [27].

4. Conclusion and Recommendation

The overall prevalence of streptococci isolated from both milks collected from the clinical and subclinical cow was 18/52 (34.6%). The prevalence of *Streptococcus* between the farm and clinical case at VTH and also among the farms were different in which CVMA farm (20%), followed by Ethiopian meat and milk institute (15%) and lower prevalence in clinical case at VTH (7.69%). The results indicated that high prevalence at the farm level which are managed intensively and at the clinic level from clinical mastitis case. Hence, poor standard hygienic and management practices inside the dairy farms which permitted the spread of both contagious and environmental infections.

4.1 Based on the above conclusion, the following recommendations were forwarded

- Cows are preventively treated with antibiotics during the dry-off period and infected cows should be rapidly detected and separated to avoid bacterial spreading
- Physical barrier that helps to prevent entrance of pathogens into the mammary gland. Should be used
- Good milking practice, such as teat disinfection and drying, as well as regular cleaning and checking of the milking machine.
- Detailed study should be conducted to investigate and identify the different groups of Streptococci

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