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Research Article

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Prevalence, Isolation and Identification of Major Bacteria Associated Goat Mastitis in and Around Haramaya Town, Eastern Ethiopia

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Abstract

The study was conducted from November 2018 to April 2019 to estimate the prevalence of sub clinical and clinical mastitis, to asses associated risk factors and to isolate the major bacterial pathogens in dairy goat in Haramaya district of Eastern Ethiopia. Among 384 lactating goats examined, 173 (45.05%) were infected with mastitis, 17 were infected with clinical mastitis and 156 were subclinical mastitis. Results of clinical examination and CMT showed clinical mastitis and subclinical mastitis. On an average 4.43% (n=17), 40.62% (n=156) and 54.95% (n=211) goats showed clinical, subclinical and negative for mastitis respectively. The highest prevalence of clinical (5.50%) and subclinical (50%) mastitis was seen at the age between 4 to 5 years and above 5 years respectively. A lowest prevalence (1.80%) of clinical and subclinical mastitis (32.10%) was seen at 2 to 3 years age group. The highest prevalence of clinical (10.50%) and subclinical mastitis (61.40%) cases were recorded at above 6th parity. The lowest prevalence of clinical (2.30%) and subclinical (36.60%) mastitis was seen at the parity between 1st and 2nd parity. The highest prevalence of clinical (6.70%) and subclinical (46.80%) mastitis was detected in goats with an early lactation period, and the prevalence rate gradually decreased as the length of lactation period was shortened. Bacteriological examination of milk sample revealed 173 clinical and subclinical cases of mastitis were E. coli, Staphylococci and Streptococci. The highest prevalence of Staphylococcus spp (n=108) was seen followed by E. coli (n=80) and Streptococci spp. The present study concluded that prevalence of mastitis particularly the subclinical mastitis was major problem of dairy goat in the area and hence warrants serious attention.

Keywords: Dairy Goat, Milk, Prevalence, Mastitis, Risk Factor, Haramaya, Ethiopia

1. Introduction

Goat population of Ethiopia ranks high both in Africa continent and the globe in general [1]. According to a recent report by Central Statistics Authority of Ethiopia, there are about 29.70 million goats in Ethiopia, of which about 71.57 percent are females and 28.43 percent are males [2].

Compared to other ruminants, goats possess unique abilities to adapt to harsh tropical environments and are closely associated with resource-poor households often found in marginal and harsh environments [3]. Their ability to adapt into different agro-ecological zones makes them the best source of milk in different regions. They can withstand high temperatures, parasites and diseases [4]. Goat production is one of the low resource demanded and efficient farming types, since goats have broad feeding habit, adaptation to unfavorable environmental conditions, low cost of maintenance, inherent suitability for small scale production and their short reproductive cycle. These provide goats with comparative advantage over cattle and sheep to suit the circumstances of especially resource poor livestock keepers [5,6].

Dairy goats are important in the rural areas where they contribute in alleviating poverty through provision of skins, meat and milk. It is also easy to keep dairy goats as the initial investment is low; they require less feed and have a good feed efficiency compared to the cow [7]. Since goats browse different variety of trees and shrubs, goat owners believed that goat milk has medicinal value for children and contribute much more for the wellbeing of human

baby [8-10]. In addition to this, goat milk provides more nutritional value than dairy cow's milk [11].

Despite the large number of goats and their contributions to the livelihood of the farmers and the national economy, goat productivity in Ethiopia is low due to different factors including shortage, seasonal unavailability, and low nutritive value of feed and/or poor nutrition [12]. Prevalence of different diseases and parasites [13,14]. One of the major diseases that affect the dairy goats is mastitis [15].

Mastitis is an inflammation of the mammary gland, caused by over 150 different contagious or environmental micro organisms [16]. In lactating dairy goats, the inflammation of the mammary gland is one of the most common infectious diseases [17]. It occurs after several pathogens invades and colonizes the secretory tissue leading to inflammation of the mammary gland [18,19].The signs of mastitis are either clinical or subclinical. In cases where there are no visible changes in appearance of milk and udder but the milk composition is altered with presence of bacteria accompanied by decreased milk production then subclinical mastitis is diagnosed [20-22]. Clinical mastitis is characterized by the visible changes in the udder and milk with the animal showing signs of anorexia and lethargy [23].

The economic loss due to mastitis has been reported to be one of the major setbacks in the dairy enterprise [24,25]. Mastitis is responsible for important economic losses and it can reduce yield and quality of the milk [26]. The economic loss is due to discarded milk, early culling, drug costs, veterinary fees, increased labor and decreased quality of manufactured milk products [27]. Therefore, mastitis is one of the important pathologies in goats with serious financial consequences [28].

There are several microorganisms that have been isolated from goats with mastitis with the commonest being bacterial infections [29]. In Ethiopia, studies show that bacterial agents such as *Staphylococcus aureus*, CNS, *Streptococcus species, Staphylococcus intermidius*, E. coli, *Pasteurella haemolytica, Pseudomonas aeruginosa, Bacillus* species, *Micrococcus* species, *Actinomyce* species, *Arcanobacterium* species, *Klebsella pneumonae* and *Enterobacter* species were frequently associated with clinical and subclinical cases of goat mastitis [30,31].

1.1 The Objective of this study is:

✓ To determine the prevalence of mastitis in goat herd kept at the study area and

✓ To isolate and identify the prevailing causal bacterial organisms from mastitis positive milk samples

✓ To assess the risk factors associated with the occurrence of different mastitis pathogens

2. Materials and Methods

2.1. Study Area

A cross-sectional study was conducted from November 2018 to April 2019 in the selected peasant association in haramaya district, eastern Hararghe zone of Oromia regional state. Haramaya district is located in the Eastern Hararghe Zone of the Oromia Region of Ethiopia, which are about 506 kilometers from Addis Ababa and 12 kilometers far from the city of Harar and 35 kilometers from Dire Dawa at an altitude of 2047 meters above sea level (m a.s.l.) between latitude 9°24′′N and longitude 42°01′′E. The mean annual rainfall is 870 mm with a range of 560 to 1260 mm and the livestock population of the study woreda is estimated to be 76 336 cattle, 65 083 sheep, 84 916 goats, 22 355 donkeys, 356 camels and 89 800 chickens [32].

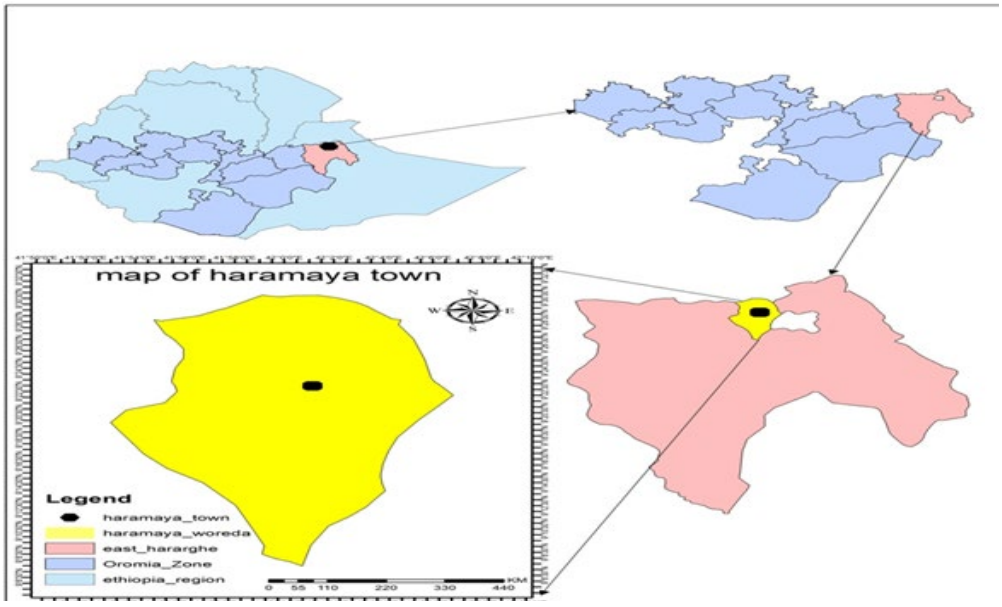

Figure 1 : Map of study area

The study animals were taken from selected peasant association of in Haramaya district which was randomly selected. In the study areas, goats are preferred animals next to cattle, and goat milk is widely consumed. The study animals were randomly selected lactating goats in the district. The predominant goat breeds in the study area are Hararghe highland breeds, which are managed under an extensive management system. Dairy goat was included into the sample regardless of age, parity, stage of lactation etc.

2.2. Study Design and Sampling Method

A cross sectional study was conducted from November, 2018 to April, 2019 to estimate the prevalence of Goat mastitis in Haramaya district. The prevalence was determined in respect to the number of risk factors such as age, parity and stage of lactation. The animals were selected by systematic random sampling technique.

2.3. Data Collection

2.4. Questionnaire

Semi structured questioner were used to assess the management and hygienic practice of lactating dairy goats. The farm owners, milking personnel and farm attendant from selected farms were face to face was interviewed on the way they handle and manage farm, milk and milk products. Generally farm/animal owner, milk collector and farm attendant were interviewed while sampling. Consequently, hygienic practices employed in the farms such as house cleaning, udder cleaning, hand washing practices and milking utensils and collecting vessels (bucket) hygiene and other condition that affects the hygienic quality of raw milk were assessed.

2.5. Physical Examination of the Udder and Milk Sample Collection

Before sample collection, the udder was examined visually and by palpation for evidence of gross pathology. Udders and teats were cleaned and dried before sample collection. The teats were disinfected with 70% alcohol before sampling. The first few streams of milk were discarded and about 15 mL of milk were collected in sterile test tubes. Milk from each goat was visually examined for evidence of physical changes. Observation of milk consistency, color changes, and presence of grossly visible substances were carried out. Milk samples were transported in an icebox with ice to the haramaya university veterinary microbiology laboratory for microbiological analysis. Clinical mastitis was recognized by some pathological changes such as swelling, pain, redness, and heat in case of acute mastitis, whereas hardening of the udder, blockage of the teats, atrophy or fibrosis, and abscess formation was regarded as chronic mastitis.

2.6. California Mastitis Test

Milk samples from each goat were subjected to CMT to test for subclinical infection of udder. Equal amount of milk and commercial reagent (California mastitis reagent) which contains 3% alky aryl sulfonate and bromocersol purple as pH level indicator were mixed in the cup on a paddle and gentle swirling were applied to the mixture in a circular motion. The result of the test was indicated on the base of gel formation [33]. The interpretation grade of CMT was evocated and the result grades 0 for negative and trace, 1, 2 and 3, for positive [34].

2.7. Bacteriological Analysis of Samples

Culture media preparation and sample inoculation: The media used to cultivate bacteria associated with sub clinical mastitis were selected and prepared based on the recommendation given by Quinn et al. [35]. Accordingly, blood agar was used for initial isolation of microorganisms from milk samples. Dehydrated media containing agar were dissolved in a hot plate which incorporate magnetic stirrer until it boils. Then the media were sterilized by autoclave at 121°C for 15min holding time, and dispensed with a volume of about 15 ml in to sterilized petri-dishes. To prepare blood agar, non-coagulated blood was collected from sheep that did not receive antibiotic therapy and gently added to molten agar base and cooled to 50°C on water bath. All CMT positive milk sample were subjected to culture on blood agar. A loop full of milk was streaked on 5% sheep blood agar plate and incubated aerobically at 37oC. The primary culture was examined for growth after 24, 48 and 72 hours of incubation to rule out slow growing microorganisms. For further identification, top 3 isolated and similar colonies were picked with sterile wire loop and transferred to other blood and MacConkey agar plates.

Identification of bacterial colony: The isolated bacterial colonies were identified based on colony characteristics such as size, shape, color, consistency, growth on MacConkey agar and hemolytic characteristics. A loop full of colony from each representative colony types were subjected to Gram's staining in order to observe Gram's reaction, cellular morphology and arrangements, catalase test, oxidase test and O-F test. After primary characterization, suspected colonies from MacConkey agar were further cultured on Eosin methylene blue agar to observe pigmentation of isolated colony. Suspected colonies from blood agar were further cultured on mannitol salt agar and subjected to coagulase test to identify pathogenic Staphylococcus species. All test protocols and means of bacterial identification were performed according to Quinn et al. [36].

2.8. Sample Size Determination

The total numbers of Lactating goat included for this study was calculated based on the formula given by Thrusfield (2005). With 95% confidential interval and 5% absolute precision and expected prevalence of 50 % is taken since there was no previous study conducted in the area.

$n= 1.962\ pexp\ (1\text{-}p_{exp})$

D2 Where; n= required sample size; P_{exp} = expected prevalence and a desired absolute precision (d) of 0.05. Accordingly, by using this formula, the required samples were 384 lactating goat.

2.9. Data Quality Control

All laboratory procedures including media preparation, procedures

of each testing technique were done according to manufacturer production guideline. Sterilization procedures and collection and handling of specimens were carried out in accordance with standard protocols [37]. The necessary reagents and samples were checked for contamination each time before handling and kept in proper condition [38].

2.10. Data Management and Statistical Analysis

The data collected was entered and scored in Microsoft excel worksheet. Before subjected to statistical analysis, the data were thoroughly screened for errors and properly coded. For analysis SPSS Microsoft software Version 20 was used. Descriptive statistical analysis was used to summarize and present the data collected. The prevalence of goat mastitis were calculated as percentage by dividing total number of goat positive for mastitis to the total number of goat examined. Prevalence of isolated pathogens was calculated as percentage by the number of positive samples divided by the total number of samples examined. Pearson chi square ($\chi 2$) test was employed to assess the existence of association between prevalence of the goat mastitis and potential risk factors considered. For ($\chi 2$) test, p-value < 0.05 were considered significant whereas p-value > 0.05 considered non-significant.

2.11. Ethical Clearance

To make this study ethically sound all the important topics in public health ethics such as consents of the participants and willingness to take part in the study was asked and acknowledged first. All the moral, cultural and religious values of the community were respected. The confidentiality of information and privacy of the participants during sample collection and interview was protected. Access to confidential records and computer files was limited by keeping records under lock and key. All of the objectivity were discussed and analyzed throughout the research.

3. Results

3.1. Clinical Examination of Udder and Cmt of Milk Samples

Among 384 lactating goats examined, 173 (45.05%) were infected with mastitis (Table 2); 17 were infected with clinical mastitis and 156 were subclinical mastitis. Clinical examination of 17 goats showed udder and teat lesions like firmness, irregular swelling, heat, pain, sensibility, or blockage of teats indicating evidence of clinical mastitis. Results of clinical examination and CMT showed clinical mastitis and subclinical mastitis. On an average 4.43% (n=17), 40.62% (n=156) and 54.95% (n=211) goats showed clinical, subclinical and negative for mastitis respectively (Table 1). The prevalence of mastitis varied depending upon the age, parity and stage of lactation (Table 3). The highest prevalence of clinical (5.50%) and subclinical (50%) mastitis was seen at the age between 4 to 5 years and above 5 years respectively. A lowest prevalence (1.80%) of clinical and subclinical mastitis (32.10%) was seen at 2 to 3 years age group. The highest prevalence of clinical (10.50%) and subclinical mastitis (61.40%) cases were recorded at above 6th parity. The lowest prevalence of clinical (2.30%) and subclinical (36.60%) mastitis was seen at the parity between 1st and 2nd parity. The highest prevalence of clinical (6.70%) and subclinical (46.80%) mastitis was detected in goats with an early lactation period, and the prevalence rate gradually decreased as the length of lactation period was shortened.

Status of mastitis	Number of goat examined	Prevalence (%)
Negative	211	54.95
Subclinical	156	40.62
Clinical	17	4.43

Table 1: Prevalence of Clinical and Subclinical Mastitis

Status	Number of positive	Overall prevalence
Mastitis	173	45.05

Table 2: Overall Prevalence of Mastitis

Parameters	Number of goat	Clinical mastitis and its percentage (%)	Subclinical mastitis And its percentage (%)	Amastitis and its percentage (%)	Pearson Chi Square	p-value
Age						
2-3 years	112	2(1.8)	36(32.1)	74(66.1)	13.414[a]	.009
4-5 years	128	7(5.5)	48(37.5)	73(57)		
>5 years	144	8(5.6)	72(50)	64(44.4)		
Parity						
Few(1-2)	175	4(2.3)	64(36.6)	107(61.1)	22.701[a]	.000
Moderate(3-6)	152	7(4.6)	57(37.5)	88(57.9)		
Many(>6)	57	6(10.5)	35(61.4)	16(28.1)		
Stage of lactation						
Early(1-2 months)	171	11(6.4)	80(46.8)	80(46.8)	10.284[a]	.036
Mid(2-4 months)	114	3(2.6)	44(38.6)	67(58.8)		
Late(>4 months)	99	3(3)	32(32.3)	64(64.6)		

Table 3 : The Prevalence of Clinical and Subclinical Mastitis Distribution by Age, Parity and Stage of Lactation of Lactating Goats

4.2. Isolation and identification of bacteria from milk samples:

Milk samples from a total of 173 clinical and subclinical cases of mastitis were attempted to grow in bacteriological medium. The bacterial isolates grown were *E. coli, Staphylococci* and *Streptococci*. Among 173 milk samples tested, 103 grew single type of bacterial colony and 70 grew mixed types. The highest prevalence of Staphylococcus spp (n=108) was seen followed by E. coli (n=80) and Streptococci spp (n=72) (Table 4).

Groups of bacteria	Frequency and percentage		
	Clinical	Subclinical	Total
Staphylococci	11 (10.19)	97 (89.81)	108 (41.54)
Streptococci	7 (9.72)	65 (90.28)	72 (27.69)
E coli	7 (8.75)	73 (91.25)	80 (30.77)
Total	25(9.62)	235 (90.38)	260

Table 4 : The Prevalence Of Various Bacteria Isolated From Clinical And Subclinical Mastitis Of Goats.

4. Discussion

The study showed the overall prevalence of clinical and subclinical mastitis in 4.43% and 40.62% cases respectively. Mugabe et al [39]. Reported a higher prevalence of sub-clinical mastitis (13.5%) than clinical mastitis (4.29%). A 10% prevalence of clinical mastitis was reported in dairy goats in Nigeria [40]. Previous studies reported the prevalence of subclinical mastitis in goats were 18.29% [41,42]. 18.64%, 24.6% [43]. 29.92% [44-46]. 36% and 38.75 the low prevalence of clinical mastitis in goats was comparable with previous reports in Pakistan, Ethiopia and in Bangladesh [47-49]. The low level of clinical mastitis may be partly associated with the fact that dairy goats with clinically observable mastitis are either treated or culled. The prevalence's of clinical mastitis in Nigeria [50,51]. And in Bangladesh were reported earlier but the prevalence appeared much higher than the present study. These observations revealed that the prevalence of caprine mastitis is not similar in various geographical regions. The difference in the prevalence of caprine mastitis could be due to the difference in rearing system, milking technique, breed consideration, environmental temperature, management of caprine mastitis etc. In this study the higher rate of caprine mastitis as seen may be due to fact that the infected goats seldom witness the therapeutic intervention and ultimately turn to sub clinical infection.

In this study there was a significant contribution of age (p=0.009), parity (p=0.000), stage of lactation (p=0.036) on caprine mastitis (CM and SCM). Haftay et al. (2016) reported a significant contribution of age (p = 0.009) onto the prevalence of subclinical mastitis. A significantly (P>0.05) associated of parity class is reported earlier (Mugabe et al., 2017). Gebrewahid et al. (2012) revealed insignificant association between risk factors and mastitis like Age (p = 0.779), parity (p = 0.201) and stage of lactation (p = 0.952). The difference in observation may be due to fact that Gebrewahid et al [52]. Carried out their study on caprine mastitis in mount region and other researchers carried out their research on plane land. Highest prevalence of clinical (5.60%) and subclinical (50%) mastitis was seen at above 5 years age group and lowest prevalence of clinical (1.80%) and subclinical (32.10%) mastitis was seen at 2-3 years age group. In this study, an increasing trend in the prevalence of clinical and subclinical mastitis was observed with the advancement of age of the goats. Higher age group (5

years or above) is epidemiologically associated with increased subclinical mastitis of goat [53]. The increased prevalence of clinical and subclinical mastitis in older animal might be due to increase length of exposure to the pathogens compared to younger animal.

An increased prevalence of CM (10.50%) and SCM (61.40%) was found at above 6th parity and lowest prevalence of CM (2.30%) and SCM (36.60%) between 1st and 2 nd parity. The prevalence of subclinical mastitis was higher in animals that were at later stage of parity e.g., at the 6th and 5th parity (Boscos et al., 1996; Razi et al., 2012). It is assumed that at the old age, there are added burden and stress onto the body due to high milk production for longer period and multiple numbers of parity. As a result immune systems of such animals are badly affected with the infectious agents leading to mastitis [54].

The present study showed that the most prevalent pathogen causing mastitis in dairy goats was Staphylococcus spp. (41.54%) followed by Escherichia coli (30.77%) and Streptococcus spp. (27.69). These results were in agreement with results from studies done in other countries. Contreras et al., [55]. Investigated bulk tank milk from commercial dairy goats in the USA and found that most of the pathogen isolated was Staphylococcus spp. (95.7%) and Ndegwa [56]. reported that milk samples from small-scale dairy goat farms in Kenya the most prevalent bacteria was Staphylococcus spp. which were 78%. Mbindyo et al., [57]. Reported that the most prevalent pathogen causing mastitis in dairy goats was Staphylococcus species (41.9%). The major mastitic pathogen present in the milk samples is Staphylococcus spp. [58,59]. Earlier, the highest prevalence of Coagulase Negative Staphylococci (CNS) was reported by Gelasakis et al, Nickerson et al. and Silanikove et al. [60-62].

5. Conclusion and Recommendations

The present study recorded an overall prevalence of subclinical mastitis was high in the study areas (54.3%) which might entail that mastitis was a major health problem of dairy cows which undoubtedly will have drawback on productivity of dairy industry and hence warrants serious attention. The current study also display that pathogenic Staphylococcal species are the major bacteria along with other environmental bacteria to be associated with sub clinical mastitis. In identified staphylococcal species, the prevalence of coagulase negative Staphylococcus (44%) was higher than the coagulase positive staphylococcus (37%). The high prevalence of staphylococcal infection detected in dairy cows has public health concern since coagulase positive staphylococcus bacteria's are capable of producing heat-stable enterotoxins, which might cause staphylococcal food poisoning outbreaks when ingested by humans in sufficient quantities. This could be an indicator of poor hygienic practices and absence of regular health monitoring of animals. Based on the above conclusion the following recommendations were forwarded [63-95].

- Regular screening for the detection of subclinical mastitis and proper treatment of cows during dry and lactation period should be practiced.
- Careful hygienic milking practice and regular health monitoring should be practiced to reduce reservoir of infection and contamination of the rest of the herd.
- Further research to identify species and strains of coagulase positive and coagulase negative staphylococcus have paramount significance to reduce and prevent the pathogen effect on dairy industry.

Declarations

Author's Contributions

All authors are equally participated, read and approved the final version of the manuscript.

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We would like to thank Haramaya University, College of Veterinary Medicine.

Ethical Approval and Consent to Participate

The protocol of my current research whose approved by college of veterinary medicine veterinary public health department of Haramaya University, Ethiopia and the ethical clearance who waived due to no major involvement of humans and animal subject welfare of ethical issues.

Competing Interests

The authors declare that they have no competing interests.

Availability of Data and Materials

The datasets used during current study are available in the manuscript.

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