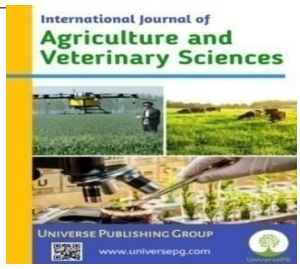
Publisher homepage: www.universepg.com, ISSN: 2663-7529 (Online) & 2663-7510 (Print)

https://doi.org/10.34104/ijavs.023.075087

International Journal of Agriculture and Veterinary Sciences

Journal homepage: www.universepg.com/journal/ijavs



Occurrence of Sub-clinical mastitis and Associated Risk Factors in Lactating Goats in Selected Areas of Eastern Harerghe Zone, Oromia, Ethiopia

Safi Hussen[1]*, Asladin Mohamed [2], and Adem Yusuf[2]

[1]Haramaya University College of Veterinary Medicine, PO. BOX 138 Dire Dawa, Ethiopia; [2]Jigjiga University College of Veterinary Medicine, PO. BOX1020 Jigjiga, Ethiopia.

*Correspondence: safihussen3618@gmail.com (Safi Hussen, Haramaya University College of Veterinary Medicine, PO. BOX 138 Dire Dawa, Ethiopia).

ABSTRACT

Subclinical mastitis reduces the quality and quantity of milk, and is a disease of great economic and public health importance. A cross sectional study was conducted to estimate the occurrence of subclinical mastitis, associated risk factors and isolate of *Staphylococcus aureus* in lactating goats in Eastern Hararghe zone, Ethiopia. A total of384 lactating goats were sampled and screened by White Side test (WST) to detect subclinical mastitis. The study results revealed that animal and udder/halve level prevalence was 23.2% and 21.8% respectively, while 30 teats were found to be blind. Univariate analysis of the potential risk factors has depicted that mastitis was more prevalent in does with medium age, poor body condition, late lactation stage, does sampled from Aweday, does mixing with others, closed housed goat, weakly manure removal and previous history of mastitis showed a statistically significant association with the proportion of subclinical mastitis ($p < 0.05$). With multivariable analysis, age, lactation stage, and origin of sampled animals showed significant association with subclinical mastitis prevalence, and these factors continued significant in multivariable logistic regression model after stepwise elimination ($p < 0.05$). As a result, does in mid age (OR=9.06, 2.24-36.60, late stage of lactation (OR=2.52, 1.21-5.24) and does sampled from Aweday (OR=4.43, 1.37-14.38) were at higher risk of udder infections than younger age, early lactation and does sampled from Haramaya separately. In the present study, *S. aureus* have been isolated from 37.1% of goat milk. Antibiogram study result indicated that gentamicin and erythromycin was found to be the most effective drug against *S. aureus*. The present study has also demonstrated the existence of alarmingly high level of antimicrobial resistances of *S. aureus* against chloramphenicol (100%), Vancomycin (87.5%) and Ampenciline (81.8%). The results of this study deep-rooted the importance of *S. aureus as* a possible causes of subclinical mastitis in goat and the spread of multiple drug resistant *S. aureus*. It is therefore, recommended to prevent subclinical mastitis in the study area by considering potential risk factors, regular screening and microbiological examination of udder of lactating goats as well as judicious use of antimicrobials to treat subclinical mastitis.

Keywords: Antimicrobial susceptibility, Lactating goats, Risk factors, Subclinical mastitis, and *S. aureus*.

INTRODUCTION:

Goats are important for livelihood of a large number of people in the third world especially to women, children, and the aged, who are the most vulnerable members of society in terms of undernourishment and poverty. Ethiopia possesses 17,000,000 goats and stands second in Africa and fourth in the world (FAO,

2012). In the eastern part of the Ethiopia goat is highly valued and reared mainly for meat and milk production which is playing an important role in the improvement of income of the poor farmers. However production is still faced by many constraints like infectious disease, scarcity of feed, high mortality rates, long marketing channels and lack of market information (Fikru and Gebeyew, 2015). Among the diseases, mastitis is considered to be one of the expensive diseases in terms of production losses (Mathew and Menon, 2008; Bardhan, 2013; Hayle *et al*., 2020).

Mastitis is any inflammation of the mammary gland or udder and one of the most important economically devastating diseases of dairy animals affecting particularly farmer in developing world (Bergonier and Berthelo, 2003). Subclinical form of the disease is imperative because it is 15 to 40 times more prevalent than the clinical form (Sawant *et al*., 2009). It adversely affects milk quality and constitutes a reservoir of microorganisms for long duration that can lead to infection of other animals (Assefa *et al*., 2014). The most common cause of subclinical mastitis in most herds or flocks is bacteria origin and other predisposing factors (Persson *et al*., 2011). In Ethiopia, the most commonly reported bacterial agents of mastitis in goat are *Staphylococcus aureus*, *Streptococcus* spp., *Corynebacterium* spp., *Escherchia coli* and *Micrococcus* spp (Assefa *et al*., 2006; Gebrewahid *et al*., 2012; Haftay *et al.,* 2016). Among these bacteria, *S. aureus* have been implicated as the leading cause of subclinical mastitis (Haftay *et al*., 2016). Diagnosis of subclinical mastitis is based on the presence of inflammatory markers in the mastitis leads to changes of milk somatic cell count (SCC) as well as chemical characteristics of milk and microbiological isolation of the causative agent (Hristov *et al*., 2015). Indirect tests such as California Mastitis Test (CMT), White Side Test (WST) and SCC are commonly used both in cows and small ruminants. White Side Test (WST) is an indirect, easily applicable screening test for sub clinical mastitis in which 4% sodium hydroxide solution are used as reagent (Contreras *et al*., 2007). Treatment and control of mastitis technique used in does is those adapted from cattle and can be udder infusion and systemic antibiotic administration. The major antibiotics used for treatment of mastitis in Ethiopia include penicillin's, streptomycin's, gentamycin and oxytetracycline. However, recent studies in country showed that there was increased resistance of *S. aureus* isolated from mastitis goat to various antimicrobial (Abdi *et al*., 2018). The main control principles include: sound husbandry practices and sanitation, post milking teat dip, treatment of mastitis during non-lactating period, and culling of chronically infected animals. Early diagnosis of mastitis with reliable tests facilitates successful treatment and control (Sharif *et al*., 2009).

MATERIALS AND METHODS:

Description of Study Area

This study was conducted in two purposively selected wored as and one administrative town of eastern Hararghe Zone namely Haramaya and Babile wored as, and Aweday town. Haramaya woreda is found in East Hararghe administrative zone of Oromia Regional state in Eastern Ethiopia. The study area has a latitude and longitude of 9°24′ N 42°01′ E and is found at an altitude of 1600-2100 meter above sea level (m.a.s.l.) with 64.5 relative humidity, is 511 km far from Addis Ababa. The woreda experience short rainy season in February and long rainy season from July to September. The annual rain fall of the areas ranges from 118-866 mm similarly the average monthly minimum and maximum temperature of the area is 9.4 and 24°C respectively. Mixed crop livestock farming is the predominant production system in the rural area. The main livestock types kept in the in study area includes cattle, sheep, goat, camel, donkey and poultry. The total livestock population of Haramaya woreda is about, 101,290 cattle, 112,354 goat, 73,846 sheep, 631 camel and 3,328 equine species (HAOR, 2019).

Babbile woreda is found in eastern Hararghe zone of Oromia Regional State. It is located 31 km away from the town called Harar and about 557 km east from Addis Ababa, the capital city of Ethiopia. It lies between 8°9'-9°23'N latitude and 42°15'- 42°53' E longitude and is characterized by semi-arid and arid climate with average annual rainfall of 410-800 mm and the annual temperature ranges from 24-28°C. It shares its border with Gursum from the north, Fedis from the west, Harari National Regional State from the north-west and Somalia National Regional State in the

east, South and South West. The total land covers an area of 200,000 hectors with a human population of 100,003. The livestock population of the woreda comprises about 76,161 cattle, 11,470 sheep, 20,644 goats, 7,393 donkeys, 15,430 camel and 21,114 poultry. Aweday town is located in the Eastern Hararghe Zone of the Oromia Region state of Ethiopia, which is about 10 kilometers far from the city of Harar and 30 km from Dire Dawa and 10 km from Haramaya university and also located at an altitude of 1600-2100 m.a.s.l between latitude 9°26' N and longitude 42°01' E. The mean annual rainfall is 870 mm with a range of 560-1260 mm, with 64.5 relative humidity and the mean maximum and minimum temperatures are 23°C and 9.25°C, respectively. The main livestock types kept in the area includes cattle, goat, sheep donkey and poultry. Local breed's goat is reared in and around the study area for meat and milk production mostly. Mixed crop livestock farming is the predominant production system in the rural area.

Study Animals and Management System

All lactating goats from selected district located in diverse geographical areas of eastern Hararghe zone Babille, Haramaya and Aweday town managed under deferent management system. Goats are often kept mixed with sheep during day and night with some possibility of keeping goats alone. The predominant goat breeds in the study area are Haraghe and Somali breeds, which are managed under intensive and extensive system. The number of goats per house hold varies considerably from two to over 20 animals per flock in some instance. Farmers who own more goats and sheep keep them in separate hose and feed /graze them while those who own few goats keep them in their own houses by tethering and feed chat left over and cut grass from field in some study. It is also common to find flocks in closed roof housed and open house. Whereas kids up to 3 months of age are separately kept around a homestead during the day time and housed separately in a specially constructed house with mud floor or may share house with people in some study area. Milking is practiced often once a day (in the morning and evening) by older woman and a single milker at a time. Milk is mainly used for household consumptions and regarded as useful for women in some study area. For this study, the average number of flock size was used to categorize the size as large or small

Study Design

A cross sectional study was conducted from May 2020 - September 2021 to determine the prevalence of subclinical mastitis in goats and their associated risk factor.

Sample Size Determination and Sampling

The sample size of lactating goats required for this study is determined according to the following formula described by Thrusfield, (2018).

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

Where,

n = sample size,

p = expected prevalence and

d = desired absolute precision at 95% Confidence level.

Thus at an expected prevalence of 50%, 5% desired absolute precision and 95% confidence level, the required sample size is calculated to be 384. One administration town and two woredas in zone namely Babile and Haramaya woredas, and Aweday town, purposively selected from 20 woredas and included in the present study. The study kebeles in the study woredas were selected using simple random sampling and goat owning households' selected using systematic random sampling based. Households from selected woreda were included in the study based on the presence of at least two lactating goat & their willingness. The calculated sample size was proportionally allocated to the respective woreda based on the estimated total target population respectively 173 from Babbile, 121 from Haramaya and 90 from Aweday based on goat. Simple random sampling was considered to select the animals when more than five lactating goat. The number of households to be selected was determined by dividing each sample size to number of lactating does and putative biological and environmental factors believed to be associated with the epidemiology of mastitis were interviewed and recorded. These include individual animal identifycation, age, stage of lactation, body conditions, housing, hygiene, service, feeding & vaccination previous mastitis history, parity & flock size recorded accordingly.

Clinical examination of udder

Animals were individually identified and clinically examined. During clinical examinations, palpation of udder and visual observation of udder lesion, clinical mastitis, udder symmetry, and size were performed. Additionally, observation of milk consistency, color changes, and presence of grossly visible substances were carried out. 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. Does showing clinical mastitis were excluded from the study whereas does with subclinical mastitis were included to this study.

Questionnaire survey

One hundred fifty owners of the examined animals were interviewed using a semi-structured questionnaire format. The questionnaire has mainly focused on herd structure, flock type (mixed or other livestock), cleaning frequency, kid management, housing type, vaccination, milking technique, knowledge of goat mastitis, importance of mastitis, treatment and general information on production, reproduction were obtained using the questionnaire for each sampling frame.

Milk sample collection

Aseptic technique was adopted in all steps pertaining to milk samples collection from does with subclinical mastists. Sterile vials were utilized in samples collection. Cotton balls dripping in 70% alcohol were also used. Cooler with ice packs was used for storing samples. Paper towels and disinfectant were used for cleaning teats (Precious *et al*., 2018). Permanent ink for labeling. About 3 ml of WST positive milk samples were put a sterile universal bottle and transported on ice box to the Haramaya university microbiology laboratory with a minimum of delay for routine culture techniques.

Laboratory Analysis of Milk Sample

White side test

This screening test was conducted according to the standard procedure recommended by Kehir *et al*., (2008). The test is performed by adding (1-2drops) of sodium hydroxide solution 0.4% to 5drops of cold milk on glass on black background and then stirring the mixture vigorously for 20 seconds and results were read. In positive reaction the milk was separate to water and shreds or flakes but in negative reaction the mixture remains uniformly opaque.

Bacteriological examination

Bacteriological examination was done on 89 WST positive milk samples according to Quinn *et al*. (2002). Briefly A loop full of milk sample streaked on blood agar base (Oxoid, UK) enriched with 5-10% defbrinated sheep blood using the quadrant streaking method and plates were incubated aerobically at 37 °C for 24 - 48 h and examined for characteristic bacterial colonies. Presumptive colonies were selected and sub cultured on selective media (MSA) and incubated aerobically at 37 °C for 24 - 48 h for color change. Pure colony sub-cultured on nutrient agar (Oxoid, UK) were identified according to their Gram reaction and morphology. Yellow color on manitol salt agar, gram-positive, catalase-positive, were presumptively identified as *Staphylococci* and subjected to coagulase test using rabbit plasma at four hours for grit of coagulation property (Quinn *et al*., 2002).

a) Gram's staining

Gram staining procedures - A colony from a 24 hour culture was emulsified in sterile distilled water and a thin smear was made on a grease free slide and was fixed by gently passing it through a flame. It was covered with crystal violet stain for 30-60 s and then the stain was washed off with slow running tap water and covered with Lugol‟s iodine for 30-60 s. The iodine was washed off with clean water and acetone alcohol was used to decolorize the smear for few seconds before washing with clean water. The smear was covered with safranin for 2 min, rinsed and air dried. The prepared slides were examined microscopically at 100x (oil immersion). Objective gram positive cocci (0.5 to 1.5 µm in diameter) that occurred singly and in pairs, tetrads, short chains and irregular grape-like clusters were suggestive of *Staphylococcus* species (Cheesbrough, 2000).

b) Manitol salt agar

The discrete colonies that confirmed by presence of clear zone of beta hemolysis were streaked on MSA plate and examined after 24-48 h incubation at 37 0C and for growth of bacteria and change of color. The presence of growth and change of pH in the media (red

to yellow color) regarded as presumptive identification of *S.aureus*. The incubated slant in nutrient agar was maintained for characterizing the isolates.

c) Catalase test

Catalase test was used to differentiate those bacteria that produce catalase, such as *staphylococci* from non-catalase producing bacteria such as *streptococci*. In this test clean slide and sterile inoculating loop were used. Small amount of organism from colony which incubated overnight was collected and placed onto the microscope slide. Then using a dropper–3% hydrogen peroxide (H2O2) dropped onto the organism on the microscope slide and observed for immediate bubble formation (O_2 + water = bubbles). Positive reactions were evidenced by bubble formation (Barrow and Feltham, 2004).

d) Coagulase test

Test was used to identify *S. aureus* which produces the enzyme coagulase which causes plasma to clot by converting fibrinogen to fibrin. The slide test were used after plasma was prepared with normal saline 0.2ml in 1.8ml of saline water then 5 ml of diluted plasma added to test tube and 0.5ml of an 18-24 hour isolated colony of bacteria from nutrient broth added and incubated at 37^0C and checked at an interval of 1, 3 and 6 hours for coagulation. Test samples that failed to react after 6 hours were left over night at normal temperature and re-examined. A positive reaction was indicated by definite clot format (Cheesbrough, 2000).

Antimicrobial Susceptibility Test

Representative isolates were examined for their *in vitro* drug sensitivity. The pure isolates in nutrient agar were first cultured in nutrient broth for 48 hrs. The sensitivity discs (DIFCO, OXIDO) were placed in inoculated Mueller Hinton agar and commercially prepared discs were placed on the plates and lightly pressed down to ensure the antibiotics disc in contact with the agar. Then incubate aerobically at 37^oC and the Inhibition zone were measured after 24 - 48 hrs on average of diameter in millimeter (Barrow & Feltham, 2004).

Commercially prepared disc used were: amoxicillin (5ug), ampciline (10ug), Gentmycine (10ug), Cholorophenicol (5ug), Erythromycin (10ug), vancomycine (30ug) and Tetracycline (30ug) which they were available in the market and subjected by owners and veterinarians in field (CLSI, 2015).

Data Management and Analysis

The collected data was checked and stored in Microsoft excel spread sheet until analysis. Both descriptive and analytic statistical analyses were performed using STATA Corp14 statistical software. Descriptive statistics involved frequency measurement and row percentage of categories of each variable. Analytical statistics comprised both univariable and multivariable logistic regression analysis.

Variables (host-related and environmental risk factors) were included in the univariable logistic regression analysis. Variables with a *p*-value <0.30 in the univariable analysis were incorporated in the multivariable logistic regression analysis. A category of a variable is said to have a statistically significant association with the CMT result (subclinical mastitis) when the obtained *p*-value is less than 5% ($p < 0.05$) or the 95% CI of the odds ratio didn't include one.

RESULTS:

Demographic Characteristics Respondents

Of the respondents ($n = 150$) participated in the study, 76 (51%) were from Haramaya, 50 (33%) from Aweday and 24 (16%) from Babile woreda. There were more female respondent in all age group than male respondent. Out of this 116 (76.7%) and 14 (23.7%) were male and female respectively with higher age above fifty years old.

Most of the respondents education level had read and write 111 (71%) followed by elementary 32 (25%) and college level 7 (4%). Averagely Higher of number of and goats per household were observed in Aweday when compared to goats in Haramaya (**Table 1**).

Table 1: Demographic characteristics of participants.

Characteristics	Categories	Respondents	
		Number	Percentage (%)
Sex	Male	34	22.7

	Female	116	77.3
Age (years)	21-30	26	17.3
	31-40	42	28
	41-50	34	22.7
	>50	48	32
Education level	Read and write	111	74
	Elementary	32	21.3
	College and university	7	4.4

Table 2: Over all prevalence of subclinical mastitis at animal levels and udder halve level.

Level	Number tested	Number positive	Prevalence (%)
Goat level	384	89	**23.2%**
Udder level	738	161	**21.8%**

Laboratory Results

Among 768 udder halves of 384 goats examined, a 7.8% (30/768) of the teats of does were blind, while 23.2% (89/384) of goats were positive for subclinical mastitis at 95% CI (0.192-0.27) and 21.8% (161/738) of teat were positive using WST below (**Table 2**).

Risk Factors

Univariate analysis

In the univariable logistic regression analysis among the potential putative risk factors age, body condition, lactation stage, origin, mixing with others, housing, frequency of manure removal and previous history of mastitis showed a statistically significant association with the proportion of subclinical mastitis ($p < 0.05$).

Whereas, parity, farming, flock size, milking technique, presence of wound and source of animal health service didn't show statistically significant association ($p > 0.05$) (**Table 3** and **4**).

Table 3: Univariate analysis of animal related risk factors for occurrence of subclinical mastitis.

Risk factor	Category	Total (N)	Number Positive	Prevalence (%)	Univariable Analysis	
					OR (95% CI)	*p*-value
	1-3 year	88	7	7.95	1.00[a]	
Age	3-5 year	172	53	30.81	5.15 (2.23-11.9)	<0.001*
	>5 year	124	29	23.39	3.53 (1.47-8.49)	0.005*
Bodycondition	Good	82	10	12.20	1.00[a]	
	Moderate and Poor	302	79	26.16	2.55 (1.25-5.19)	0.010*
	Early	97	14	14.43	1.00[a]	
Lactation stage	Mid	124	24	19.35	1.42 (0.69-2.92)	0.337
	Late	163	51	31.29	2.70 (1.40-5.20)	0.003*
	Few	186	50	26.88	1.00[a]	
Parity	Moderate	114	20	17.54	0.58 (0.32-1.04)	0.065
	Many	**84**	**19**	**22.62**	**0.80 (0.43-1.46)**	**0.458**

Table [a] Reference; CI: Confidence interval; *Significant

Table 4: Univariate analysis of managmantal risk factors for occurrence of subclinical mastitis.

Risk factor	Category	Total (N)	Number Positive	Prevalence (%)	Univariable Analysis	
					OR (95% CI)	*p*-value
	Hramaya	121	16	7	1.00[a]	
District	Aweday	90	34	37.78	3.98 (2.02-7.84)	<0.001*
	Babille	173	39	22.54	1.91 (1.01-3.61)	0.046*
Management System	Extensive	146	33	22.60	1.00[a]	

	Intensive	238	56	23.53	1.05 (0.65-1.72)	0.835
Flock size	<10 goats	266	63	23.68	1.00[a]	
	≥ 10 goats	118	26	22.03	0.91 (0.54-1.53)	0.724
	Alone	123	18	14.63	1.00[a]	
Mixed with other	Mixed with sheep	261	71	27.20	2.18 (1.23-3.85)	0.007*
Type of House	Open	268	54	20.15	1.00[a]	
	Closed	116	35	30.17	1.71 (1.04-2.81)	0.034*
	Daily	59	6	10.17	1.00[a]	
Manure remove	Weekly	61	17	27.87	3.41 (1.24-9.40)	0.018*
	Irregular	264	66	25.00	2.94 (1.21-7.16)	0.017*
Milking	Squeezing	375	86	22.93	1.00[a]	
	Pulling	9	3	33.33	1.68 (0.41-6.86)	0.470
History of mastitis	No	72	8	11.11	1.00[a]	
	Yes	312	81	25.96	2.81 (1.29-6.10)	0.009*
Wound	Absent	349	80	22.92	1.00[a]	
	Present	35	9	25.71	1.16 (0.52-2.59)	0.709
Service	Veterinaria	78	15	19.23	1.00[a]	
	Animal health	306	74	24.18	1.34 (0.72-2.49)	0.356
Vaccine	Vaccinated	101	19	18.81	1.00[a]	
	Not vaccinated	283	70	24.73	1.42 (0.80-2.50)	0.227

Table [a] Reference; CI: Confidence interval; *Significant

Multivariate analysis

From eleven variables with categories p-value < 0.30 and entered in the multivariable analysis, Age, lactation stage and origin of sampled goat were found to be significantly associated with the prevalence of SCM in the final multivariate analysis and found to be independent explanatory variably in does subclinical mastitis occurrence. Studied does between 3-4 age were at more risks for udder infections than those in other age group (OR=9.06; 95% CI= 2.24-36.60). Similarly, does in late lactation stage were about three times at more risk than animals does in early lactation stage (OR=2.52; 95% CI=1.21-5.24) and animals sampled from Aweday (OR=4.43; 95% CI= 1.37-14.38) had higher odds (about 4.4 times higher) of being subclinical mastitis positive than those sampled from Haramaya (**Table 5**).

Table 5: Multivariate analysis of risk factors associated with subclinical mastitis.

Risk factor	Category	Total (N)	Number Positive	Prevalence (%)	Univariable Analysis	
					OR (95% CI)	*p*-value
	1-3 year	88	7	7.95	1.00[a]	
Age	3-5 year	172	53	30.81	9.06 (2.24-36.60)	0.002*
	> 5year	124	29	23.39	0.52 (0.11-2.45)	0.410
Lactation stage	Early	97	14	14.43	1.00[a]	
	Mid	124	24	19.35	1.48 (0.66-3.45)	0.341
	Late	163	51	31.29	2.52 (1.21-5.24)	0.014*
	Haramaya	121	16	13.22	1.00[a]	
District	Aweday	90	34	37.78	4.43 (1.37-14.38)	0.013*
	Babille	173	39	22.54	0.64 (0.19-2.22)	0.484

Table [a] Reference; CI: Confidence interval; *Significant

Bacterial Isolation

During the course of the study a total of 89 milk samples were collected from does with subclinical mastitis and cultured on blood agar media. Accordingly, growth of different groups of bacteria was observed in 78.6% (70/89) of milk samples. However, since the objective was to isolate *S. aureus*, further bacteriological tests were conducted by taking only

those colonies presumptive of this species and leaving others. Of the total 89 examined goat milk samples, 33 (37.1%) were positive for *S. aureus.* Though the highest number of *S. aureus* were isolated from Babile goat milk (48.7%) followed by Aweday (29.4%) and Haramaya (25%), the prevalence of *S. aureus* from subclinical mastitis samples was not significant ($p > 0.05$) among these three study site (**Table 6**).

Table 6: Summary of bacterial culture results by origin of goats.

Origin	Total (N)	*S. aureus* Prevalence		
		Number Positive (%)	95% CI	p-value
Aweday	90	10/34 (29.4)	0.4 - 2.2	
Haramaya	121	4/16 (25)	1.10 – 12	0.671
Babile	173	19/39 (48.7)	0.4 - 2.3	

Antimicrobial Susceptibilities of Isolates

Almost all 33 *S. aureus* isolates were subjected to antibiotic susceptibility tests. Six different antibiotics were used in this test. In-vitro antimicrobial resistance pattern of *S. aureus* isolates to the six antibiotics has been investigated. *S. aureus* were found to be resistant to chloramphenicol (84.8%) vancomycine (87.9%) and ampicillin (81.8%). The isolate were also moderately resistant to amoxicillin (48.5%) and tetracycline (42.4%). *S. aureus* were found to be susceptible to gentamicin (75.8%) and erythromycin (72.7%) (**Table 7**).

Table 7: Antimicrobial sensitivity profile of *Staphylococcus aureus* strains ($n = 33$; #: Number).

Antimicrobial Agent	Disc Content	# Susceptible (%)	# Intermediate (%)	# Resistant (%)
Amoxicillin	30 µg	17/33 (51.5)	-	16/33 (48.5)
Ampicillin	10 µg	6/33 (18.2)	-	27/33 (81.8)
Chloramphenicol	30 µg	5/33 (15.2)	-	28/33 (84.8)
Erythromycin	10 µg	24/33 (72.7)	3/33 (9.1)	6/33 (18.2)
Gentamycin	10 µg	25/33 (75.8)	5/33 (15.2)	3/33 (9.1)
Tetracycline	5 µg	17 /33(51.5)	2/33 (6.1)	14/33 (42.4)
Vancomycin	5 µg	4/33 (12.1)	-	29/33 (87.9)

DISCUSSION:

In this study goat farming was found to be practiced small-scale farmer by both sexes. However there were more female 116 (77.3%) than male 34 (23.7%). This outcome was in agreement with result of Ogola *et al.* (2010) and Ngugna, (2017) who report that goat farming in Kenya was practiced by female than male farmer. However the current result was contrast with result of (Mbndyo *et al*., 2014; Fikru and Gebeyew, 2015; Gebreyowhens and Kumar, 2017) who report that goat farming was practiced by male than female farmer in eastern Ethiopia, Kenya and Nigeria. The reason why there was more female got farmer than male is due to the fact that goat was easily managed by female unlike cattle. Different age group was participating in goat farming in both sexes. However large proportion of respondent's age (41.2%) was 41-50 and above fifty. This result was in agreement with Byaruhanga *et al.* (2017) who found similar age of respondents participate in goat farming. The study explained that goat production in the study area was a mature older aged business and suggested that older age farmers were engaged in goat farming. This could be attributed to the fact that tendering of the goats under tethering system, which was predominant in study area, requires less attention, so, it was easier for older people to manage goats with this system. In this study the overall does level prevalence of subclinical mastitis was 23.2% (95% CI, 19-27). This finding was close agreement with the previous finding of other researchers (15.5%) in South Ethiopia (Megersa *et al.* 2010), 18.3% in North parts of Ethiopia (Gebrewahid *et al*., 2012) and 24% in Southern Ethiopia (Ahmed *et al*., 2019). However present finding were lower than another study reported in Oromiya region (40.9%) (Assefa *et al*., 2014), Kenya (61%) (Mbndyo *et al*., 2014) and Tanzania (51.5%) (Swai *et al*., 2001). The variation in prevalence with those result observed in other areas might be due to difference in diagnostic tools, management systems of study animal and as

well as genetic variation in disease resistance among breeds and technical knowledge of the investigators. Goats aged > 2 - 4 years had higher prevalence (30. 81%) of subclinical mastitis compared with goats aged between 1-2 years and >4 with occurrence rates of 7.9% and 23.39%, respectively. Satirical analysis reveal that there was significant association ($p<0.05$) which was in line with study of (Ameh and Tari, 2000; McDougall *et al*., 2002; Moroni *et al*., 2005). But in contrast with that of Gebrewahid *et al*. (2012).The increase in occurrence of mastitis with age may be due to the fact that older goats are long term caretakers that have stayed longer in the herd and have higher chance exposure to pathogenic infection. In the present study, there was positive association between the body condition of goats and prevalence of subclinical mastitis. This result was close agreement with report of (Koop *et al*., 2009; Megersa *et al*., 2010; Mungube *et al*. 2004) who prove that low body condition was the major risk factor for increased mastitis prevalence, but showed no statistically significance association. Lacttion stage was the major risk factor for increased mastitis prevalence and factor remained significant in the final model. This finding was in agreement with result of Miblu, (2007) and Moroni *et al*. (2005) who reported that early stage and later stage of lactation had more infection than mid stage of lactation. Nevertheless there were contrast with finding of (Ndgwa *et al*., 2001; Gebrewahid *et al*., 2012; Mbindiyo *et al*., 2014) who found no association between occurrences of subclinical mastitis with lactation stag of doe. Higher prevalence during late stage of lactation could be linked to imbalance energy intake and decline immunity. High content of somatic cell in milk at late stage increase false positive in screening. In the present study, there was increasing prevalence of subclinical mastitis with increasing parity number of lactating goats which was in line with pervious study by (McDougall *et al*., 2002; Megersa *et al*., 2010; Gebrewahid *et al*., 2012). Nevertheless there were contrast with finding by (Ndgwa *et al*., 2001; Gebrewahid *et al*., 2012; Mbindiyo *et al*., 2014) who found no association between occurrences of suclinical mastitis with lactation stag of doe. This is credited due to the health care of does and parturition stress. Untreated case in the first parity takes natural course to chronic status possibility of carryover of infection from the first parity to the next may occur. In this study, goats that were sampled from Aweday had a higher prevalence of subclinical mastitis (37.8%) compared with goats sampled from Haramaya and Babbile with prevalence of 13.2% and 22.5% respectively. This increase occurrence in Aweday may be due to management system. Since all the goats sampled from Aweday were kept in closed house and intensive management system where as goats sampled from Haramaya were smaller in size and good in hygienic standard when compared with the other locations from where the goats sampled. In this study prevalence of sub clinical mastitis were higher in does kept in closed house (30.17%) and does mixed with sheep (27.20%) when compared with studied does kept in open house (20.15%) and alone (14.63%) respectively. which was closed agreement with study result of (Ndegwa *et al*., 2000; Megarsa *et al*., 2010; Aqib *et al*., 2018) who found high occurrence of sub clinical mastitis in studied animal in closed house with earth floor type and animal reared with sheep but showed no statistically significant association. This increase occurrence of subclinical mastitis in animal kept in closed house and animal mixed with other could be explained by the fact that muddy wet earthen floors is a common finding in most closed housed and mixed rearing system which tends to harbor a wide range of infectious agents that may contaminate the udder and teats. In this study, there was significantly higher prevalence of mastitis in does whose houses were cleaned irregularly and weekly when compared to those whose house were cleaned more frequently but statistically not significant. These results were consistent with study result of (Bergonier *et al*., 2003; Mbindiyo *et al*., 2014; Precious *et al*., 2018). Current prevalence of *S. aureus* was 37.1% which was higher than the previous reports of (Molla *et al*., 2006; Gebrewahid *et al*., 2012) who report 12.8% and 27% prevalence in the southern and northern parts of Ethiopia. The high prevalence of *S. aureus* in this study might be associated with poor hygienic conditions were pathogens originate from does environment that infect the udder via the teat canal since dirty and wet bedding is a common earthen floors, tends to harbor a wide range of infectious agents that contaminate the udder and teats. The purpose of antimicrobial test is to generate information about which drug is effective against *S. aureus* causing

goat subclinical mastitis in the study area. The evaluation of the antimicrobial susceptibility *S. aureus* isolated from goats with subclinical mastitis to decide which antibiotics should be administered, as well as, for monitoring the spread of multiple resistant strains on farmhouses (Salvatore *et al*., 2010).

In this study isolated *S. aureus* showed high percent of antimicrobial resistance to chloramphenicol, vancomycine and ampicillin. These results were in line with the results of Haftay *et al*. (2016) who observed a resistivity of 100% to vancomycine, 90.9% to ampicillin in a study carried out in northern Ethiopia; and Onanuga *et al*., (2005) and Aqib *et al*., (2018) who also found a 100% and 70% resistance to chloramphenicol in Southern Nigeria, and Pakistan respecttively. However the present study was not concurs with earlier study by Teshome *et al*., (2015) who obtained *S. aureus* isolates were found to be resistant with tested antibiotics in the following percentage: chloramphenicol (10.5%), vancomycine (5.5%). The probable explanation to the presence of high antibiotic resistant by *S. aureus* to chloramphenicol and vancomycine may be due to indiscriminate and protracted/ repeated use of these antibiotics in human health and indiscriminate use of antibiotics by individual farmers without prescription of professionals in study area. *S. aureus* has been known to show multiple antimicrobial resistance patterns (Alian *et al*., 2012). The widespread use of antibiotics has undoubtedly accelerated the evolution of *S. aureus*, which as a result of the acquisition of multiple resistance genes has become able to survive almost all antibiotic families (Alian *et al*., 2012). In the present study *S. aureus* isolates also high susceptibility to erythromycin, gentamycin tetracycline and amoxicillin which was comparable with the results obtained by Tashom *et al*. (2013) who found *S. aureus* isolates sensitive for the tested antibiotics in the following percentage: Erythromycin (78.9%), gentamycin 84.2%, tetracycline 56.2% and amoxicillin 57.9%. However, (Tofaily *et al*., 2011; Mekuria *et al*., 2013) found that the susceptibility rate of *S. aureus* isolates to Erythromycin was 21.6% and 16.6%. This might be that these antibiotics are not frequently used in the study area in veterinary services and possibly in human medicine. Studies show that susceptibility patterns of *S aureus* to antimicrobial agent have varied worldwide, but isolates were usually susceptible to gentamicin (Alian *et al*., 2012).

CONCLUSION AND RECOMMENDATIONS:

Subclinical mastitis of lactating does in eastern Hararghe was encountered condition. *S. aureus* was a prevalent bacteria isolated from does with subclinical mastitis; this indicated that *S. aureus* was an important cause of subclinical mastitis. Risk factors, such as age, stage of lactation and the study site were responsible factors for the occurrence of subclinical mastitis in the study area. *S. aureus* isolates were developed resistance to various antimicrobial agents. Resistance to Vancomycin and chloramphenicol has been recorded in almost all *S. aureus* isolates and needs serious attention. However *S. aureus* isolates were susceptible for erythromycin and gentamycin. These all findings may provide essential information on epidemiology of the disease and the causal agents to implementing an integrated prevention and control strategy of goat subclinical mastitis in the study area.

The following recommendations are made based on the key findings of this study.

- Additional investigations of other pathogens involved in goat mastitis will optimize our knowledge of causative agents and control interventions
- Microbiological examination of udder of lactating goats at regular intervals should be routinely carried out in order to detect subclinical mastitis and underline course.
- Judicious use of antibiotics may be included in designing in the prevention strategies against occurrence of SCM with antibacterial resistant isolates.
- In-vivo antimicrobial susceptibility patterns of *S. aureus* isolates should do.

Ethical Approval

Ethical approval was obtained from the Institutional Research Ethics Review Committee (IHRERC) of Haramaya University, College of veterinary medicine official letter of support was sent to SGS office and to other concerned bodies. This study was conducted in accordance with the Declaration

ACKNOWLEDGEMENT:

We are thankful to veterinary laboratory personnel and university authorities to the completion of the successful research study.

CONFLICTS OF INTEREST:

The authors declare that there is no conflict of interest.

REFERENCES:

1) Abdel, (2019). Body Condition Scoring of Local Ossimi Ewes at Mating and its Impact on Fertility and Prolificacy. *Egyptian J. of Sheep & Goat Sciences*, **4**: 37-44. https://www.researchgate.net/publication/273886955
2) Ahmed Badaso, Barbara Wieland and Bekele Megersa. 2019. Mastitis in Lactating Cows, Camels and Goats in Borana, Southern Ethiopia. *J. of Science & Development,* **7**, 2-9.
3) Alian F, Rahimi E, and Momeni M, (2012). Antimicrobial Resistance of *Staphylococcus aureus* Isolated from Bovine, Sheep and Goat Raw Milk. *Glob. Vet*, **8**, 111-114. https://www.researchgate.net/publication/268005889
4) Ameha, A., Tari, L.S., (2000). Observation on the prevalence of caprine mastitis in relation to predisposing factors in Maiduguri. *Small Rumin Res,* **35**, 1-5.
5) Aqib, A.I., Farooqi, S.H. and Hussain, K., (2017). Identifcation of coagulase gene in *Staphylococcus aureus* isolates recovered from subclinical mastitis in camels. *Pakistan Vet. J*., **37**, 160-164.
6) Assefa Wakwoya, Bayleyegn Molla., and Goetz Hildebrandt., (2006). A Cross-Sectional Study on the Prevalence, Antimicrobial Susceptibility Patterns, and Associated Bacterial Pathogens of Goat Mastitis. *Intern J Appl Res Vet Med*, **4**, 169-76.
7) Assefa, E., Yosef, T. and Berhanu, G. (2011). Goat production system and opportunities for market orientation. *Tropentag*, **1**, 1-4. https://www.slideshare.net/ILRI/goat-production
8) Bardhan, D., (2013). Estimates of economic losses due to clinical mastitis in organized dairy farms. *Indian J. of Dairy Science,* **66**, 168-172
9) Barrow, G., and Feltham, R.K.A., (2004). Cowan and Steel's Manual for the Identification of Medical Bacteria. *Cambridge University Press, UK*
10) Bergonier, D., Berthelot, X. (2003). New advances in epizootiology and control of ewe mastitis. *Livest. Prod. Sci*, **79**, 1-16.
11) Byaruhanga, C., Oluka, J. and Olinga, S., (2014). Socio-economic aspects of goat farming enterprise in Teso region, Uganda. *Uganda J. of Agricultural Sciences*, **15**, 87- 100.
12) CLSI, (2015). Performance standards for antimicrobial susceptibility testing. Clinical & Laborator Standard Institute. *Document, Wayne, PA*, pp. M100-S124. https://clsi.org/standards/products/microbiology/documents/m100/
13) Contreras, A., Paape, M. J and Gonzalo, C. (2007). Mastitis in small ruminants. *J. of Small Ruminant Research,* **68**,145-153.
14) FAO, (2012). (Food and Agricultural Organization) Crop and Food Security Assessment Mission to Ethiopia. Special report. *Food and Agriculture Organization of the United Nations, Rome, Italy*.
15) Fikru S, Gebeyew, K, (2015). Sheep and Goat Production Systems in Degehabur Zone, Eastern Ethiopia: Challenge and Opportunities. *J. Adv Dairy Res,* **3**,134. https://doi.org/10.4172/2329-888X.1000134
16) Gebrewahid, T., Abera, B., and Menghistu, H. (2012). Prevalence and Etiology of Subclinical Mastitis in Small Ruminants of Tigray Regional State, North Ethiopia. *Vet World*, **5**, 103-109.
17) Gebreyowhens and Kumar, R. (2018). *Inter. J. of Livestock Production,* **9**(3), 50-66
18) Haftay, A., Habtamu, T.M., and Abebe, M.S. (2016). Bacterial identification and antimicrobial susceptibility of subclinical mastitis causing bacteria from goats in Aba‟lla district, Afar, North-Eastern Ethiopia. *Revue Méd. Vét*, **167**, 7-8.
19) HARO, (2019). Haromaya agricultural research organization. Beef Research Strategy. *Animal Science Directorate.*
20) Hristov, K., Parvanov P., and Nikolov, B., (2015). Prevalence of mastitis and dynamics of health status mammary gland during lactation and dry period in goats. Scientific Works. Series. *J. of Veterinary Medicine*, **4**, 163 -167.
21) Hayle WA, Ahmed R, and Uddin ME. (2020). Prevalence of subclinical mastitis among small

ruminants and isolation of some bacterial pathogens in Jimma Town, Ethiopia, *Eur. J. Med. Health Sci*., **2**(6), 107-124. https://doi.org/10.34104/ejmhs.020.01070124

22) Kahirl, M.D., Siddiqur, M.D., and Jong, S., (2008). Prevalence and risk factors of subclinical bovine mastitis in dairy farm of Sylhet district of Bangladesh. *Korean, J. Vet Service*, **31**, 497-l4.

23) Koop, G., Bacon, D. and Gardner, I. A., (2013). Risk factors for subclinical intramammary infection in dairy goats in two longitudinal field studies evaluated by Bayesian logistic regression. *Prev. Vet. Med*, **108**, 304-312. https://doi.org/10.1016/j. prevetmed.2012.11.007

24) Mathew, L and Menon, D.G., (2008). Economic impact of FMD in Chazhoor Panchayath, *Veterinary World*, **1**, 5-6.

25) Mbindyo, C. M., Gitao, C. G, and Bebora, L. (2014). A cross-sectional study on the prevalence of subclinical mastitis and antimicrobial susceptibility patterns of the bacterial isolates in milk samples of smallholder dairy goats in Kenya. *American J. of Research Communication,* **2**, 8-12.

26) McDougall, S., Pankey, W., and Scruton, D., (2002). Prevalence and incidence of subclinical mastitis in goats and dairy ewes in Vermont, USA. *Small Ruminant Researc,* **46**, 115-121.

27) Megersa Tadesse, Abunnaregassa, Mekibib and Debela, E. (2010). Occurrence of mastitis and associated risk factors in lactating goats under pastoral management in Borana, Southern Ethiopia. *Trop. Anim. Hlth. Prod,* **42**, 1249-55.

28) Mekuria, A., Asrat, D., and Tefera, G., (2013). Identification and antimicrobial susceptibility of *Staphylococcus aureus* isolated from milk samples of dairy cows and nasal swabs of farm workers in selected dairy farms around Addis Ababa, Ethiopia. *Afr. J. Microbial*. Res, **7**, 3501-3510. https://doi.org/10.5897/AJMR12.2060

29) Mibilu, K., (2007). Status of mastitis in, lactating goats at Sokoine University of Agriculture and neighboring small holder farms in Morogoro Municipality, *Tanzania Livestock. Rural Development,* **19**, 54-60.

30) Molla B, Wakwoya A, and Hildebrandt G., (2006). A Cross Sectional Study on the Prevalence, Antimicrobial Susceptibility Patterns, and Associated Bacterial Pathogens of Goat Mastitis in Adami Tulu Oromia Ethiopia, *Int. J. Appl. Res. Vet. Med*, **4**, 169

31) Moroni, P., Ruffo, G. and Carli, S. (2004). Antibiotic susceptibility of coagulase-negative staphylococci isolated from goat's milk. *Int. J. Antimicrob. Agents*, **23**, 637-640. https://doi.org/10.1016/j.ijantimicag.2003.10.007

32) Mungube, E.O., Tenhagen, B.A., and Baumann, M.P.O., (2004). Risk factors for dairy cow mastitis in the central high land of Ethiopia. *Trop. Anim. Health Prod*, **36**, 463-472.

33) Ndegwa, E.N., Mulei, C. M. and Munyua, S. J., (2000). The prevalence of subclinical mastitis in dairy goats in Kenya, *J. S. Afr. Vet. Assoc*., **71**, 25-27.

34) Ndegwa, E.N., Mulei, C.M. and Munyua, S.J.M. (2001). Risk factors associated with subclinical sub-acute mastitis in Kenyan dairy goats, *Israel J. Vet. Med,* **56**, 4-28.

35) Nugugna, (2017). Cross - Sectional study on prevalence of mastitis and associated risk factor In Dairy Goat in Small Scale Farm in Kenya. Faculty of Veterinary Epidemiology and Economics School of Graduate Studies M.Sc thesis in university of Kenya.

36) Ogola, T. D., Nguyo, W. K., & Kosegey, I.S., (2010). Dairy goat production practice in keniya. Implication for breeding program. *J. of livestock research for rural development*, **22**, 20-25.a

37) Onanuga, A.O., Oyi, A. H. and Onaolapo, J.A. (2005). Prevalence and susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolates among healthy women in Zaria, Nigeria. *African J. of Biotechnology*, **4**, 1321-1324. https://www.ajol.info/index.php/ajb/article/view/71404

38) Persson, Y. and Olofsson, I. (2011). Direct and indirect measurement of somatic cell counts as indication of intrammamary infections in dairy goats, *J. of Acta Veterinaria*, **60**(1), 53- 15

39) Precious. M., Naomi, M. and John K., (2018). Prevalence, Risk Factors, and Antibiogram of Bacteria Isolated from Milk of Goats with Subclinical Mastitis in Thika East Subcounty, Kenya, *J. of Veterinary Medicine*. p.8 https://doi.org/10.1155/2018/3801479

40) Quinn, P.J., Donnelly, W. J and Leonard, F.C., (2002). Veterinary microbiology and microbial diseases. 1st Edition. *Blackwell Science Ltd*., PP: 175-179.

41) Salvatore, V., Carlo, S. and Enrico Pietro, L.D.S., (2010). Antibiotic Resistance in *Staphylococcus aureus* and Coagulase Negative Staphylococci Isolated from Goats with Subclinical Mastitis *Veterinary Medicine*. Article ID 517060, 6 pages https://doi.org/10.4061/2010/517060

42) Sawant, A.A., Gillespie, B. E. and Oliver, S. P., (2009). Antimicrobial susceptibility of coagulase negative Staphylococcus species isolated from bovine milk. *Vet. Microbiol.,* **134**, 73-81.

43) Sharif, A and Muhammed, G. (2009). Mastitis control in dairy animals. *Pakistan Veterinary J.*, **29**, 145-148.

44) Swai, E.S, Mobies, E., and Mtui, P.F. (2008). Occurrence and Factors Associated with Udder Infections in Small Holder Lactating Dairy Goats in a rumeru District, Tanzania. *Livestock Res. Rural Dev*, **12**, 1-12.

45) Teshome, B., Genene, T., and Abebe, M. (2016). Prevalence and antimicrobial susceptibility pattern of *Staphylococcus aureus* from raw camel and goat milk from Somali region of Ethiopia. *Afr. J. Microbial*. Res, **10**, 1066-1071. https://doi.org/10.5897/AJMR2015.7517

46) Thrusfield, M.V. (2007). Veterinary Epidemiology. Third Edition, Blackwell Science Ltd, 9600 *Garsington Road, Oxford UK*.

47) Tofaily, Y.I., Kha L.M, and Alrodhan, A.N., (2011). Study on Clinical Mastitis (Bacteriological) in She-Camels (*Camelus dromedarius*) in Some Areas of Middle Euphrates in *Iraq. J. Vet. Med. Sci*, **10**, 2-51. https://doi.org/10.29079/vol10iss2art156

Citation: Hussen S, Mohamed A, and Yusuf A. (2023). Occurrence of sub-clinical mastitis and associated risk factors in lactating goats in selected areas of Eastern Harerghe zone, Oromia, Ethiopia. *Int. J. Agric. Vet. Sci.*, **5**(4), 75-87. https://doi.org/10.34104/ijavs.023.075087