

Prevalence and risk factors of sub-clinical mastitis in goats in Bangladesh



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Table of contents

Cover Page.....	I-II
Acknowledgments.....	VI
Abstract.....	01
1. Introduction.....	02-03
2. Materials and Methods.....	04-06
2.1 Study area and study population.....	04
2.2 Data collection.....	04
2.3 Collection and transportation of milk samples.....	04
2.4 Milk sampling.....	04
2.5 Bacteriological culture.....	05
2.6 <i>Staphylococcus aureus</i>	05
2.7 Statistical evaluation.....	05
2.7.1 Descriptive analysis.....	06
2.7.2 Univariate analysis.....	06
3. Results.....	07-11
3.1. Descriptive analysis.....	07
3.1.1. Demography of goat farmers.....	07
3.1.2. Characteristics of sampled goats in SAQTVH at CVASU.....	08
3.1.3. Management features of goat farms in Chattagram metropolitan city.....	08
3.1.4. Isolated organisms and the status of California Mastitis Test.....	09
3.2. Risk factor analysis of sub-clinical mastitis in goat in relation to California Mastitis Test.....	10
3.2.1. Univariate Analysis.....	10
3.3. Risk factor analysis of sub-clinical mastitis in goat in relation to organisms	10
3.3.1: Univariate association of different factors in correspondence to binary response variables of <i>S. aureus</i> at animal level.....	11
4. Discussion.....	11-13

4.1. Descriptive association of the status of California Mastitis Test and different bacterial organism.....	11
4.2. Discussion on risk factor analysis of sub-clinical mastitis in goats in relation to California Mastitis Test.....	11
4.3. Discussion on risk factor analysis of sub-clinical mastitis in goats in relation to <i>S.aureus</i>	12
4.4. Farmers' and farms' demography and management traits of goat farms.....	12
4.5. Limitations of the study.....	13
5. Conclusions.....	14
6. References.....	15-16
7. Biography.....	17

List of Abbreviation

CVASU	Chattogram Veterinary And Animal Sciences University
SAQ TVH	Shahedul Alam Quadary Teaching Veterinary Hospital
SCM	Subclinical Mastitis
Co NS	Coagulase Negative Staphylococcus
CMT	California Mastitis Test
SCC	Somatic Cell Count
MS Excel	Microsoft Excel
BCS	Body Condition Score
AI	Artificial Insemination

List of Table

Table 3.1: Goat farmers’ demographic information in CVASU, SAQ TVH (N=100).....	07.
Table 3.2: Characteristics of sampled goats in Chattagram metropolitan city (N=100).....	08
Table 3.3: Management features of goat farms in Chattagram metro city (N=100).....	09
Table 3.4: Univariate association between selected factors and California Mastitis Test status (Yes/No) at animal level of lactating goats in SAQ TVH ,CVASU	10
Table 3.5: Univariate association between selected factors and <i>S. aureus</i> test status (Yes/No) at animal level of lactating goats in CVASU.....	10

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Abstract

Goats are a major source of supplementary income for rural populations in Bangladesh and play a significant role in the country's economy. The demand for healthy food is driving up interest in dairy goats and goat milk products since goat milk is less allergic than cow milk and has nutritional properties that are comparable to those of human milk. The fact that the number of goats in Bangladesh has increased over the past 20 years is another indication of this significance. Mastitis, a bacterial infection of the mammary gland, is the primary cause of poor milk quality. Lactating goats are among the dairy animals that are most susceptible to intramammary infections, which can have a significant financial impact on farmers. However, there are minimal scientific studies present on caprine subclinical mastitis in Bangladesh. The present study was undertaken to determine the proportionate prevalence of sub-clinical mastitis along with the potential risk factors associated with sub-clinical mastitis and mastitis related organisms such as *Staphylococcus aureus*. The teat and udder of 100 lactating goats were physically examined.

Most of the goats were reared under intensive as well as semi-intensive rearing systems. Wooden floor was more common floor type among the studied farm and others used earthen floor and some were found using only plastic and clothing bags over mud. The cross-breed goats were reared more than the other breeds like beetal, jamunapari and local.

In this study the major bacterial pathogens isolated was *Staphylococcus aureus* (13%). Chi-squared test was applied to identify potential risk factors associated with SCM in goats. Teat end shape (pointed, rounded and flat) and Age (minimum 32 months versus maximum 33 months) were identified as the risk factors for SCM in goats. Knowledge obtained from the study could help in practicing hygienic management systems for goat farming to reduce SCM in goats.

Key words: Sub-clinical Mastitis, Goat, Risk factors, Prevalence, Chattagram

Chapter I: Introduction

Mastitis is a severe problem for lactating does in Bangladesh. Goats and other animals frequently suffer from subclinical mastitis (SCM), an inflammatory condition of the mammary gland that does not manifest as typical signs of clinical mastitis, such as pain, swelling of the udder, or changes in the appearance of milk. This subtle disease can seriously impair the health and productivity of animals, resulting in lower milk yield and quality, a higher chance of developing clinical mastitis, and financial losses for farmers. It is found in animals raised for meat and dairy purposes. Any animal that produces milk including sheep, goats and cows can develop mastitis which is an inflammation or infection of the mammary gland or udder. Several pathogenic agents, chiefly microorganisms that infiltrate the mammary gland triggering off an inflammatory response, are responsible for the development of SCM. The age, breed, management style, and environmental conditions of the goat can all affect the specific causative agent that causes SCM.

In Bangladesh, sub-clinical mastitis (SCM) is a serious health issue that costs dairy goat farmers a lot of money. The difficulties with sub-clinical mastitis on dairy farms have caused the goat farmers to lose interest in maintaining their animals. Sub-clinical mastitis infections have an impact on dairy products by lowering milk yield, compromising health, and impairing fertility (Salum et al., 2022). In goats of northern Bangladesh, the frequency of SCM was 56.2%, (Begum et al., 2016). Overall, a recorded average prevalence of 30.0% was observed for SCM (Moni et al., 2020).

Although *Staphylococcus* sp. are thought to be the main agents that cause mastitis and are responsible for clinical and SCM (De Visscher et al., 2017), bacteria such as coagulase-negative Staphylococci (CoNS), coliforms, and *Streptococcus* sp. can all induce sub-clinical mammary gland inflammation, or SCM (Akter et al., 2020). In dairy goats, coagulase-positive *Staphylococcus aureus* is the most frequently identified bacterial pathogen associated with clinical mastitis, while coagulase-negative *Staphylococcus* spp. is more frequently discovered in sub-clinical mastitis cases (Mishra et al., 2014). SCM can result in decreased milk production and quality as well as an increased probability of clinical mastitis although in the absence of clinical symptoms (Adkins et al., 2018).

Several reasons, such as source, California Mastitis Test (CMT) score, parity, housing system, floor materials, and management systems, have been found to be risk factors for SCM in goats. As compared to Black Bengal goats, Jamunapari goats are more susceptible to SCM (Akter et al., 2020).

Therefore, the current study aimed to quantify the prevalence of SCM along with identifying reasonable risk factors that have been associated with SCM and find out the cause of SCM.

Objectives of the study:

1. To assess socio-economic status of goat farmers and to find out the goat farm characteristics with its management traits in Chattogram metro city.
2. To determine potential risk factors associated with sub-clinical mastitis and mastitis associated organisms.

Chapter II: Materials and methods

2.1. Study area and study population

A cross sectional study was conducted in SAQTVH (S. A. Quaderi Teaching Veterinary Hospital) at CVASU. The reference population included all goats on household farms that were brought to the SAQTVH from various locations within the Chattagram metropolitan area. Only lactating goats were considered a source population in the SAQTVH. To cover the various goat rearing zones in the Chattagram metropolitan area, a total of 100 lactating goats were selected. Following that, these goats were used in a cross-sectional study design to collect samples. All the lactating goats from different farms enrolled were sampled, resulting in N = 100 goats.

2.2. Data collection

A structured questionnaire was used to collect information regarding the owners and farms demography ; traits and management in a face to face interview. Data collection based on udder health, teat shape, any defects in udders and also teat end shape of individual lactating goats was done. The next step was to collect individual data from a goat that had been diagnosed with SCM (subclinical mastitis) in order to examine the relationship between various risk factors with the identified organism.

2.3. Collection and transportation of milk samples

All udder quarters of the 100 lactating goats were sampled in this study. A total of 10 ml milk sample per quarter was collected aseptically and placed in a 15 ml sterile falcon tube with a unique identity number. All samples collected were immediately transported using an insulated ice box to the laboratory at CVASU. Samples were stored at -20°C before further analysis.

2.4 Milk sampling

Each individual goat underwent the California Mastitis Test to check for subclinical mastitis. 2 ml milk and 2 ml of CMT reagent were put to the CMT paddle after discarding a few squirts of foremilk. The test result was then evaluated and scored from 1 to 5. The somatic cell count (SCC) results from the CMT were graded

and interpreted as 0, negative (healthy quarter), 1+, weak positive (SCC >100,000-500,000 cells/ml milk), 2+, distinct positive (SCC >500,000-1000,000 cells/ml milk), and 3+; strong positive (SCC 1000,000 cells/ml milk). SCM was considered to be positive with a score of 2 or higher. A goat was judged to be positive for SCM if the sample received a score of 2 or higher on the CMT examination. For bacteriological analysis, the teats of the goat were disinfected with 70% ethyl alcohol before milk collection and nearly 5 ml of milk was collected in 15 ml sterile falcon tubes with a specific identification number. All samples were brought to the lab at Chattogram Veterinary and Animal Sciences University in Bangladesh as soon as possible using ice boxes. Prior to additional bacteriological study, samples were kept at -20 °C.

2.5. Bacteriological culture

A bacterial culture was done on 100 samples of milk. To observe the growth of several types of bacteria, culturing was done in a petri dish with agar media. The agar medium was incubated for 24 hours at 37 degrees Celsius. Every agar plate was evaluated for the best bacterial colony growth. Certain kinds of isolates were ignored because they were thought to be laboratory contamination from undesirable species cultures. To detect whether a gram-positive cocci is a staphylococci or a streptococci, the catalase test was applied. Then Another method to distinguish the highly pathogenic *S. aureus* from the other less pathogenic *Staphylococcus* sp. is a coagulase test.

2.6 Staphylococcus aureus

The following characteristics were considered for identification of *S. aureus* were golden yellow colony with beta hemolysis on blood agar, yellow colonies with yellow zones on mannitol salt agar, grey white to yellow colonies on nutrient agar, gram positive cocci in cluster in grams staining, catalase positive and coagulase negative.

2.7 Statistical evaluation

Field and laboratory data were stored in the spreadsheet of Microsoft Excel (MS) 2007 programme. Data were cleaned, coded, recorded and checked for integrity in MS Excel 2007 before exporting to STATA-IC-13 (*Stata Crop, 4905, Lakeway Drive, College station, Texas 77845, USA*) for performing epidemiological analysis.

2.7.1 Descriptive analysis

Descriptive analysis was performed on the data of farmers' demography (frequency distribution), farms' demography, characteristics of sampled goats and management features of farm as well as organisms (frequency distribution). The result was expressed as frequency number and percentage where applicable.

2.7.2 Univariate analysis

Source of goat, floor materials of farm, BCS, parity, lactation period and teat shape were initially assessed by Chi-squared test to identify univariate association between the status of SCM and the selected factors. The level of significance of the test was set at $p \leq 0.05$.

Chapter III: Results

3.1. Descriptive analysis

3.1.1. Demography of goat farmers

Goat farmers had a diverse profession in this study; such as Businessman (52.08%), Housewife (16.67%), Job (15.63%), Driver (11.46%), Online Business(1.04%), Farmer(2.08) , Van driver(1.04%)

Table 3.1: Goat farmers' demographic information in Chattogram (N=100)

Variable	Category	Frequency number	%
Profession	Businessman	30	34.1
	Online business	11	12.5
	Housewife	22	25.0
	Farmer	9	10.2
	Job	11	12.5
	Driver	5	3.7
	Van Driver	13	14.8

3.1.2. Characteristics of sampled goats in SAQTVH at CVASU.

Multiple goat breeds were recorded in the study area where cross bred was (74%) local was (9.0%) , Jamunapari was (8%) and Beetal goat was (5%). About 49% goats had the first parity followed by 47% the second to third parity , 1 % more than the third parity and 2% absent . The BCS of goats was very thin (47%), thin (52%) and moderate (1%). (Table 3.2).

Table 3.2: Characteristics of sampled goats in Chittagong metropolitan city (N=100)

Variable	Category	Frequency number	%
Breed	Beetal	5	5
	Jamunapari	8	8
	Local	9	9
	Cross	74	74
Parity	Absent	2	2
	First	49	49
	Second to third	47	47
	More than third	1	1
Body Condition	Very thin(2)	47	47
Score	Thin(3)	52	52
	Moderate(4)	1	1
Right teat end shape	Flat	17	17
	Pointed	28	28
	Rounded	54	54
Left teat end shape	Rounded	56	56
	Flat	19	19
	Pointed	24	24
Symptoms	Swollen mammary gland	19	19
	Absent	81	81

3.1.3. Management features of goat farms in Chattagram metropolitan city

The farm floor was made of clothing bags (7.0%), Earthen floor (18%), plastic (14%) and wood (61%). Goats were generally reared intensive (97 %) and others semi intensive (3%). Goats breeding naturally (54%) and AI (45%). Majority of farmers have no knowledge about mastitis (73.74%) where a few farmers have knowledge about mastitis (26.26%) (Table 3.4).

Table 3.3: Management features of goat farms in Chittagong metro city (N=100)

Variable	Category	Frequency number	%
Floor materials	Clothing bag	7	7
	Earthen floor	18	18
	Wood	61	61
	Plastic	14	14
Housing	Semi intensive	3	3
	intensive	97	97
Breeding	AI	45	45.6
	Natural breeding	54	54.6
Any knowledge about mastitis	No	73	73.7
	Yes	26	26.3

3.1.4. Isolated organisms and the status of California Mastitis Test

The prevalence of organism in relation to CMT was calculated based on animal level. The prevalence of *Staphylococcus aureus* is 13%. The bacteria was isolated by culture media with some significance tests such as catalase and coagulase tests.

3.2 Risk factor analysis of sub-clinical mastitis in goat in relation to California Mastitis Test

3.2.1. Univariate Analysis

The proportionate prevalence at animal level varied significantly by right teat end shape, left teat end shape, Age (months) ($p \leq 0.25$). When comparing goats with pointed right teat ends to those with rounded, flat right teat ends, prevalence of SCM was significantly higher (85.71%) in the former group ($p \leq 0.000$). Furthermore goats having pointed left teat end shape had significantly greater levels of SCM (91.67%) than those of the goats whose left teat end shape is rounded and flat (35.71% and 15.79% respectively) ($p \leq 0.000$). Sub-clinical mastitis was more commonly affected in goats with an age of maximum 33 months (62.50%) than in goats having age minimum 32 months (37.04%) ($P=0.023$).

Table 3.4: Univariate association between selected factors and California Mastitis Test status (Yes/No) at animal level of lactating goats in Chittagong metro city

Factor	Category	N	CMT status		p (Chi-square d test)
			Positive (%)	Negative	
Right teat end shape	Pointed	28	24(85.71)	4	0.00
	Rounded	54	15(27.78)	39	
	Flat	17	3(17.65)	14	
Left teat end shape	Pointed	24	22(91.67)	2	0.00
	Rounded	56	20(35.71)	36	
	Flat	19	3(15.79)	16	
Age (months)	Min/32	27	10(37.04)	17	0.02
	33/max	72	45(62.50)	27	

N: Number; CMT: California Mastitis Test; p: Probability

Table 3.5: Univariate association between selected factors and *S. aureus* test status (Yes/No) at animal level of lactating goats in Chittagong metro city

Factor	Category	N	S. aureus		p (Chi-square d test)
			Positive (%)	Negative	
Source	Farm borne	12	6(50.00)	6	0.000
	Bought	88	7(7.95)	81	
Symptomp	Absent	81	8(9.88)	73	0.055
	Swollen mammary gland	19	5(26.32)	14	

N: Number; S.aureus: *Staphylococcus aureus*; p: Probability

3.3 Risk factor analysis of sub-clinical mastitis in goat in relation to organisms

3.3.1: Univariate association of different factors in correspondence to binary response variables of *S. aureus* at animal level.

Intramammary infection due to *S.aureus* was significantly higher in farm borne (50.00%) in contrast to goats which are bought (7.95%) (p=0.000). *S.aureus* was more in goats showing symptoms of swollen mammary gland (26.32%) than in goats which are not showing any symptoms (9.88%) , (p=0.055).

Chapter IV: Discussion

Subclinical mastitis poses a significant financial challenge in the dairy sector, notably affecting goat farms. Limited research conducted in Bangladesh indicates the potential susceptibility of these farms to subclinical mastitis, emphasizing the economic impact of this condition. Thus, the current study was conducted in SAQTVH at CVASU to evaluate the status of sub-clinical mastitis and the risk factors and pathogen distribution associated with it, as well as the socioeconomic characteristics of farmers and the characteristics of their farms and management practice. This section of the study has discussed important findings of the current study and their implications along with limitations, conclusions and recommendations.

4.1. Descriptive association of the status of California Mastitis Test and organism

The overall proportionate prevalence of SCM estimated as 55% by CMT in the goat population in SAQTVH at CVASU in the current study which is supported by number of studies conducted in northern districts of Bangladesh (56.2%) (Begum et al., 2016) and 20 to 50 % reported by Bergonier et al. (2003).

According to a previous report, *S. aureus* is the cause of up to 40.0% of mastitis cases in China and other nations (Kateete et al., 2013; Basanisi et al., 2017; Wang et al., 2018). *S. aureus* was found in 47.97% (n = 83) of cows in sub-clinical mastitis (Rana et al.,2022). A study found that *S. aureus* was the most commonly identified pathogen from the 827 quarter cases of clinical mastitis (24%)(Neijenhuis et al., 2001).

4.2. Discussion on risk factor analysis of sub-clinical mastitis in goat in relation to California Mastitis Test

The anatomical shape of teat end was one of the important risk factors for being positive in CMT in this study. The right teat end shaped with pointed were (85.71% higher risk of being CMT positive than rounded and flat right teat end shape (27.78% and 17.65% respectively). Teat-end shapes showed some association with SCM (P = 0.07) (Singh et al., 2017). Additionally, cows with clinical mastitis had more often pointed teat ends than non-mastitis cows (6 versus 2%; P = 0.019).(Neijenhuis et al., 2001)

According to a study, some teat morphological characteristics in Frieswal crossbred cows are linked to SCM; as a result, selecting for positive morphological characteristics may help lower the incidence of mastitis in this breed (Singh et al.,2017)

The present study showed that results of goats aged maximum to 33 months could be the reason for the increased SCM . It was determined that early lactation and advanced age were potential risk factors that combined with SCM.(Fazal et al.,2023)

4.3. Discussion on risk factor analysis of sub-clinical mastitis in goat in relation to *S. aureus*

Subclinical mastitis (SCM), an often asymptomatic inflammatory condition of the mammary gland in goats, is frequently associated with *Staphylococcus aureus* (*S. aureus*), a ubiquitous bacterial pathogen.

Goats with Subclinical mastitis, attributed to *S. aureus*, poses challenges due to its asymptomatic nature. *S. aureus* is a common mastitis-causing pathogen with the ability to persist in the udder, making early detection difficult. The lack of visible signs makes it imperative for goat farmers to employ regular milk quality testing. Factors such as inadequate milking hygiene and contaminated bedding contribute to *S. aureus* transmission, emphasizing the importance of a clean milking environment. The prevalence rate of *S. aureus* in raw goat milk responsible for subclinical mastitis was found to be 25.58% in Siliragung Subdistrict, Banyuwangi District, East Java, Indonesia.(Praja et al., 2023)

4.4. Farmers' and farms' demography and management traits of goat farms

Regardless of types, most of the goat farmers are businessmen (34.1%) with 12.5% online business 25% housewife , 10.2% farmer ,12.5% job holders ,3.7% driver and 14.8% van driver in the present study.

Intensive and semi-intensive goat rearing systems are commonly practiced among the study animals . where intensive rearing is 97% . The present study identified that the floor of the goat house was made of wood (61.0%), clothing bag (7.00%), plastic (14%) and earthen floor (18%). A wooden floor is easy to make compared to other types of floor, especially floor having plastic and clothing bag materials ,and also earthen floor.

This observation could be explained by the fact that farm management is related to intramammary infection or mastitis. Preventing diseases in the herd is essential for the health of the animals as well as the economy. Hygiene and protective measures should be implemented before the disease reveals itself in the herd.

(Gökdağ, et al., 2020)

4.4 Limitations of the study

- i. Sample size: The study was conducted in 100 goats only. The sample size was small which might have introduced lower statistical power and bias on the reliability of estimates in the study.
- ii. Sampling techniques: The animals under the study were chosen from CVASU's SAQTVH. As a result, the study may have had selection bias due to the sampling methods, which may not have accurately represented the population it was taken from. The study's external validity may be compromised by the practical sampling strategy.
- iii. Recall bias during information recording: The farmers' responses to various questions were recorded using a structured questionnaire as part of the study. Recall bias may have been introduced by the response, which could have overestimated the odds or vice versa and affected the study's findings.
- iv. Diagnosis bias: The California Mastitis Test was used to diagnose the sub-clinical mastitis in the goat. Therefore, inadequate CMT sensitivity and specificity may introduce bias into the diagnosis made during laboratory testing, leading to an underestimate or an overestimation of the actual prevalence of sub-clinical mastitis in the study population.

Chapter V: Conclusions

5.1. Conclusion

Goats were brought to SAQ TVH and CVASU, where Staphylococci, the primary cause of SCM, are highly prevalent. The origin of goats, the type of breeds , and the shape of the teat end were linked to the incidence of SCM. Control programs are necessary to improve the health of Bangladeshi goats' udders, and they should concentrate on educating farmers about mastitis and helping them choose breeds that have better udder and teat characteristics.

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Biography

I am Zannatul Mawa . I was born in Chattogram District . I have passed my Secondary School Certificate (SSC) examination from Chattogram Cantonment High School, Chattogram in 2014 and Higher Secondary Certificate (HSC) examination from Chattogram Cantonment Public School and College, Chattogram in 2016. I enrolled for Doctor of Veterinary Medicine (DVM) degree in Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh in the 2016-2017 session. In the near future, I would like to make my career in veterinary field practice.