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# Prevalence of Caprine Subclinical Mastitis in Mymensingh Area, Bangladesh and Characterization of Associated Bacterial Agents and the Risk Factors

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**A B S T R A C T**

The prevalence of caprine subclinical mastitis in Mymensingh area was assessed by California Mastitis Test (CMT). Milk samples were also analyzed for the isolation and identification of bacterial agents that might be associated with caprine subclinical mastitis including their antibiotic sensitivity pattern against commonly used antibiotics. In addition, the influence of certain risk factors such as age of animal, parity, length of lactation period, type of farming system and type of housing floor, on the prevalence of caprine subclinical mastitis was also evaluated. Milk samples were collected from 59 goats (113 udder halves). The overall prevalence of caprine subclinical mastitis as determined by CMT was 18.64%. On an udder half basis the prevalence was 15.04%. Certain risk factors associated with caprine subclinical mastitis were identified. The prevalence was higher in older animals, with greater parity and longer lactation period. The prevalence was also higher in farms where goats were raised under traditional farming system with earthen floors. The predominant bacterial species isolated from milk that were collected from the subclinical mastitis positive cases were *Staphylococcus aureus, S. epidermidis* and *Bacillus subtilis*, that showed various degrees of sensitivity to the antibiotics used in the study.

**Keywords:** Subclinical mastitis, Caprine, Prevalence, Bacteria, Risk factors



## Introduction

Mastitis refers to the inflammation of the udder. Mastitis is an important livestock disease and under untreated conditions, it constitutes a serious problem in dairy herds with considerable economic consequences, mainly due to fall in milk production, decreased milk quality for dairy purposes and poor milk hygiene; specially important when unpasteurized milk is used for cheese production (Seegers *et al.*, 2003; Persson and Olofsson, 2011). In subclinical mastitis, there are no obvious clinical signs such as abnormal milk, udder swelling or tenderness, or systemic signs such as fever, depression. Instead there is an increase in somatic cell counts of the milk (Radostis *et al.*, 1999).

Goats are one of the major sources of meat and dairy products in many areas of the world (Haenlein, 2004). In the last few years, an increase in the global goat population has been reported by FAO (2010). There are about 21.60 million goats in Bangladesh (DLS, 2009) and the domestic goat farming has secured an important source of income for many farmers in this country. Goat milk production and processing worldwide is only about 2% of total milk production from all dairy animals combined (goats, sheep, buffaloes and cattle). However, goat milk production is of major economic importance in many countries including Bangladesh (55% of all milk), Somalia (51%), Mali (43%), Indonesia (29%), Greece (26%), Iran (24%), Sudan (16%) and Algeria (13%) (Haenlein, 1996). According to recent FAO (2010) report, Bangladesh remained in the top position for producing goat milk globally as it reached 2,168,000 tons in 2009. Therefore, any factor such as mastitis that adversely affects the quantity and quality of goat milk is of great financial interest in the goat rearing countries of the world including Bangladesh.

Several procaryotic and eucaryotic pathogens and predisposing factors have been identified that are associated with the onset of mastitis in goats (East *et al.*, 1986; Contreras *et al.*, 1995). In addition, several risk factors such as milking hygiene, management practice, stage of lactation and parity are also known to have influence on the occurrence of mastitis in goats (East *et al.*, 1986; Boscos *et al.*, 1996). While clinical mastitis is rather easy to detect, animals suffering from subclinical mastitis are often very

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difficult to find since there is a lack of reliable diagnostic methods, especially at farm level (Leitner *et al.*, 2004). However, two indirect tests *viz.* somatic cell count and bacterial load count are accepted reliably for detecting the early infection (Schalm *et al.*, 1971). In addition, other indirect tests like White Side Test and California Mastitis Test (CMT) have been developed for rapid screening of udder infection (Schalm *et al.*, 1971; Guha *et al.*, 1989). If the cases are not detected in time, subclinical mastitis may progress and develop into clinical cases (Adwan *et al.*, 2005). Therefore, a rapid detection of udder health and the knowledge of risk factors are the basic requirements for the outcome of treatment to prevent the loss of milk production as well as prevent the spread of infection. Although many developing countries have proper strategies for this disease monitoring and control, there is little information available regarding the prevalence of subclinical mastitis in goats in Bangladesh. Therefore, the present study was carried out to determine the prevalence of subclinical mastitis in goat in Mymensingh and to identify and characterize the associated bacterial agents and the risk factors.

## Materials and Methods

### Study area

The present research work was carried out on Local and Crossbreed lactating goats from Goat and Sheep Breeding Farm at Bangladesh Agricultural University (BAU), and local farm around the BAU campus, Mymensingh during the period of August 2010 to April 2011.

### Collection of data and the milk samples

A total of 113 udder halves milk samples were collected from 59 animals. No milk was available in other 5 udder halves. Milk samples were collected by soaking the teat with Povidone Iodine and drying off by disposable tissue paper. Initially, one to two drops of milk was discarded and then 5 ml of milk was taken from each udder halves into sterilized test tubes with rubber cap. Each sample was labeled with an identification number. Data were also collected on age and parity of animals such as - how many times the animal gave birth to offspring, length of lactation period, type of farming system and type of floor in the farm (Table 2).

### Determination of subclinical mastitis

Subclinical mastitis was determined by CMT using a CMT kit (Leucocytest®, Synbiotics Corporation-2, Alexander Fleming -69007 Lyon, France) according to the instruction of the manufacturers. The CMT reagent reacts with DNA of epithelial and inflammatory cells present in the milk. CMT results were read immediately and were scored for each teat depending on the amount and thickness of gel formed. In this study, CMT scores of '0' and 'trace' were considered as negative or normal while CMT scores of 1+ (weak positive), 2+ (distinct positive) and 3+ (strong positive) were taken as indicators of subclinical mastitis.

### Isolation and identification of bacterial agents

Isolation of bacterial agents from the milk samples were carried out by culturing the milk samples on blood agar, McConkey agar, eosin methylene blue agar and nutrient agar plates. The inoculated plates were incubated at $37^0$C under aerobic condition for 24-48 hours. Identification of the bacterial agents from the pure culture were carried out based on their colony characteristics, Gram staining reaction, hemolysis pattern and biochemical test as described by Merchant and Packer (1967) and Cheesbrough (1985).

### Antimicrobials sensitivity tests

Among the isolated bacterial agents, five isolates of *Staphylococcus aureus, S. epidermidis* and *B. subtilis* were selected randomly for the antibiogram study. Antibiotic sensitivity test of the selected bacterial isolates was performed by Disc Diffusion test according to the method described by Bauer *et al.* (1966). This method allowed the rapid determination of *in vitro* efficacy of an antibiotic by measuring the diameter of the zone of inhibition, which results from diffusion of the agent into the medium surrounding the disc. Ten commercially available antibacterial agents (Oxoid Ltd., UK) were selected for the purpose. The name of these antibacterial agents, their concentration per disc and the diameter of zone of inhibition that was used to interpret the data are presented in Table 1.

**Table 1. Antibacterial agents used for the determination of antibiotic sensitivity pattern**

| Antibacterial agents | Concentration (µg/disc) | Interpretation of results (Zone diameter in mm) | | |
|---|---|---|---|---|
| | | R | I | S |
| Tetracycline | 30 | ≤14 | 15-18 | ≥19 |
| Streptomycin | 10 | ≤11 | 12-14 | ≥15 |
| Amoxicillin | 10 | ≤11 | 12-14 | ≥15 |
| Chloramphenicol | 30 | ≤12 | 13-17 | ≥18 |
| Cephalexin | 30 | ≤11 | 12-15 | ≥17 |
| Ciprofloxacin | 5 | ≤15 | 16-20 | ≥21 |
| Gentamicin | 10 | ≤12 | 13-14 | ≥15 |
| Nalidixic acid | 30 | ≤14 | 15-17 | ≥18 |
| Cephradine | 30 | ≤12 | 13-15 | ≥16 |
| Kanamycin | 30 | ≤12 | 13-14 | ≥15 |

µg = Microgram; mm = Millimeter; S =Sensitive; I = intermediately sensitive; R = Resistant

### Statistical analysis

The main categorical variables of the analysed goats were classified in agreement with animals' age, parity, length of lactation period, farming system and type of floor. The prevalence of subclinical mastitis was determined using the positives and the total number of analyzed goats in each of the aforementioned variables (Table 2). In addition, the association between categorical variables and the presence of subclinical mastitis positive goats was carried out using a stratified cross-sectional design and calculating the adjusted prevalence ratio (PR). This analysis was obtained with the Win Episcope 2.0 (Veterinary School, Zaragoza, Spain) at a 95% level of confidence (Table 2).

## Results

### Prevalence of caprine subclinical mastitis

In this study, among the 59 goats that were tested for the prevalence of subclinical mastitis by CMT, 11 were positive (18.64%). Udder half prevalence was 15.04% (Table 2). The association between the prevalence of subclinical mastitis was positively correlated with age. The prevalence of subclinical mastitis varied depending on the parity of the animal and length of their lactation period. The highest prevalence (66.66%) was detected both at $6^{th}$ and $5^{th}$ parity, whereas lowest prevalence (4.76%) was found at $2^{nd}$ parity, and the $4^{th}$, $5^{th}$ and $6^{th}$ were considered as risk factors (Table 2). The highest prevalence of subclinical mastitis was detected in animals with a lactation period of 3-4 months, and the prevalence rate gradually decre-

**Table 2. Prevalence of subclinical caprine mastitis based on various parameters**

| Parameters | | No. of goat or sample tested | No. of positive cases (Prevalence in %) | Prevalence ratio (PR) | *P* value |
|---|---|---|---|---|---|
| Total animal tested | | 59 | 11 (18.64) | | |
| Milk samples (Quarter) | | 113 | 17 (15.04) | | |
| Age | 2-3 Year | 34 | 4 (12.50) | 0.33 | <0.0001 |
| | 3-4 Year | 20 | 5(25.00) | 1.22 | |
| | 4-5 Year | 5 | 4 (80.00) | 4.80 | |
| Parity | 1st | 9 | 1 (11.11) | 0.56 | <0.0001 |
| | 2nd | 21 | 1(4.76) | 0.18 | |
| | 3rd | 16 | 1(6.25) | 0.27 | |
| | 4th | 7 | 4 (57.14) | 4.25 | |
| | 5th | 3 | 2(66.66) | 4.15 | |
| | 6th | 3 | 2(66.66) | 4.15 | |
| Length of lactation period | 0-1 month | 17 | 0 (0) | 0 | 0.009 |
| | 1-2 month | 38 | 9 (23.68) | 2.49 | |
| | 2-3 month | 3 | 1 (33.33) | 1.87 | |
| | 3-4 month | 1 | 1 (100) | 5.80 | |
| Farming system | Traditional system | 33 | 8 (24.24) | 2.10 | <0.0001 |
| | Semi-intensive system | 26 | 3 (11.53) | 0.48 | |
| Type of floor | Slatted floor | 22 | 3 (13.63) | 0.63 | <0.0001 |
| | Concrete floor | 4 | 0 (0) | 0 | |
| | Earthen floor | 33 | 8 (24.24) | 2.10 | |

Here: If PR = 1, then there is no statistical association between disease and variables
If PR > 1, then there is a positive association between disease and variables (risk factor)
If PR < 1, then there is a negative association between disease and variables (protective factor)

ased as the length of the lactation period shortened (Table 2). However, the lactation length of 0-1 month was not associated with subclinical mastitis, while all the other lactation lengths were considered as risk factors (Table 2).

In this study, the prevalence of caprine subclinical mastitis was found to be influenced by farming system and type of floor used to raise the goat. The prevalence was higher, 24.24%, and considered a significant risk factor in farms where goat was raised under a traditional farming system compared to 11.53% in semi-intensive farming system. The prevalence was also higher (24.24%) in farms that had earth floor type compared to farms with slatted floors (13.63%). Also, only the earthen floor housing was significantly associated with subclinical mastitis. None of the animals from a farm that had a concrete floor was found positive for subclinical mastitis.

**Isolation and identification of bacterial agents**

Seventeen milk samples from positive cases of subclinical mastitis were examined for the isolation and identification of bacterial agents. Among these 17 milk samples, 5 (29.41%) were found positive for *S. aureus.* In addition, 3 samples (17.64%) were positive for both *S. epidermidis* and *B. subtilis* (Table 3). Although most cases were associated with single infection, mixed infections with *S. aureus* and *S. epidermidis* or Gram-negative rod along with *Bacillus* sp. and a coccal pathogen were detected in few cases.

**Antibiotic sensitivity pattern of the bacterial agents**

The antibiogram study revealed that the isolated *S. aureus* were highly sensitive to amoxicillin, chloramphenicol and tetracycline and resistant to nalidaxic acid and streptomycin. Isolated *S. epidermidis* showed varying degrees of sensitivity to all the antibiotics used in this study with highest sensitivity to amoxicillin followed by tetracycline and ciprofloxacin. All the *B. subtilis* showed varying degrees of sensitivity to all the antibiotics used in this study with highest sensitivity to amoxicillin, ciprofloxacin, chloramphenicol and tetracycline. None of *S. epidermidis* and *B. subtilis* was resistant to any of the antibiotics used in this study.

**Table 3. Prevalence of bacterial agents isolated from caprine subclinical mastitis milk samples**

| Bacterial isolates | No of positive cases | Prevalence (%) |
|---|---|---|
| *Staphylococcus aureus* | 5 | 29.41 |
| *Staph. epidermidis* | 3 | 17.64 |
| *Bacillus subtilis* | 3 | 17.64 |
| *S. aureus* + *S. epidermidis* | 4 | 23.52 |
| Gram-negative rod + *Bacilli* + Cocci | 2 | 11.76 |

## Discussion

In this study, the overall prevalence of subclinical mastitis was 18.64% by CMT in the goat population in Mymensingh area. On a udder half basis, the prevalence was 15.04%. The prevalence of caprine subclinical mastitis in other countries such as in Brazil is 22.5% (Schmidt *et al.*, 2009), in Pakistan is 13% (Ali *et al.*, 2010) and in Ethiopia is 18.03% (Gebrewahid *et al.*, 2012). Recently, Islam *et al.*, (2011) determined the prevalence of caprine subclinical mastitis in Savar area, Dhaka. The authors reported 34% prevalence of subclinical mastitis compared to 18.64% found in the present study. This observed variation in the prevalence of caprine subclinical mastitis in Bangladesh could be related with different managemental practices and two different locations of the study areas. The CMT was shown to be useful way for screening goats for the presence of increased somatic cell count and presence of an intramammary infection. However, at population level, false negative CMT results were observed (McDougall *et al.*, 2010).

Age of animal was always been an important factor that govern the prevalence of subclinical mastitis in goat (Boscos *et al.*, 1996; Sharma *et al.*, 2007; Ali *et al.*, 2010). In the present study, a trend in increase in the rate of prevalence of subclinical mastitis was observed as the age of the animal increased (Table 2). Higher age (3 years or above) was found epidemiologically associated with caprine subclinical mastitis. Our present findings support the earlier observations of Sharma *et al.* (2007) and Ali *et al.* (2010), who also observed an increased prevalence rate of subclinical mastitis in comparatively older goats. This

increased prevalence of subclinical mastitis in older animal might be due to increased length of exposure of older animal to pathogens compared to younger animal. In addition, Ali *et al.* (2010) suggested that older animal are under stress resulting from long time milk production and multiple numbers of parturitions. As a result, such animals are easily become the host of infectious agents due to low immunity.

In the present study, the prevalence of subclinical mastitis was influenced by the parity of the animal and the length of lactation period. The prevalence of subclinical mastitis was higher in animals that were at later stage of their parity e.g., at the 6$^{th}$ and 5$^{th}$ parity, as reported earlier by Boscos *et al.* (1996). Sánchez *et al.* (1996) also reported parity as the risk factor for subclinical intramammary infection in goats. The highest prevalence of subclinical mastitis was also observed in animal that had a lactation period for 3-4 months.

Prevalence of caprine subclinical mastitis also appeared to be influenced by farm management system such as type of farming system and type of floor used to raise the goat in the studied area. In this study, the prevalence of subclinical mastitis was higher in farms where goat was raised under traditional conditions and had an earthen floor (soil surface). Earthen floor has been reported to be an important risk factor for subclinical mastitis in goat (Ndegwa *et al.*, 2000). This observation could be explained by the fact that dirty and wet bedding, which was a common finding on the earthen floors, tends to harbor a wide range of infectious agents, which may contaminate the udder and the teats. Based on the findings of this study, we suggest to use semi-intensive farming system with either slatted floor or concrete floor to raise goats in order to reduce the prevalence of subclinical mastitis as these conditions appeared to be a protective factor (Table 2).

In the present study, among bacterial agents isolated from caprine milk samples that were found positive for subclinical mastitis, *S. aureus* was the most dominant species. About 29.41% of the milk samples were positive for *S. aureus.* The antibiogram study revealed that most of the isolated bacterial species were sensitive to various antibiotics used in the study at varying levels except the *S. aureus*, which was found resistant to nalidixic acid and streptomycin. All the tested isolated bacterial species were found highly sensitive to broad spectrum antibiotics such as amoxicillin, chloramphenicol, ciprofloxacin and tetracycline suggesting that these antibiotics could be the first choice of drug for the treatment purpose. Many authors reported antibiotic resistant strains of *S. aureus* as the dominant bacterial species frequently isolated from milk samples collected from subclinical caprine mastitis (Mhase *et al.*, 2007; Ali *et al.*, 2010; Gebrewahid *et al.*, 2012). In addition, Islam *et al.* (2011) found *S. aureus* as the major etiological agent for subclinical mastitis in goat in Savar area of Bangladesh. *S. aureus* is probably the most infectious agent because it causes a chronic and deep infection in the mammary glands, which is extremely difficult to cure (Miles *et al.*, 1992). Presence of *S. aureus* in the milk samples also has public health significance, since many of the *S. aureus* produce various enterotoxins that could result in food poisoning (Fagundes *et al.*, 2010). Moreover, these *S. aureus* were found to be resistant to antibiotics thus making them more potential threats for farmers who are in close contact with these animals, since disease caused by them could be very difficult to treat. Due to their high contagious nature (Fox and Gay,1993), *S. aureus* could also easily be transmitted from infected animal to healthy animal through direct or indirect contact to cause disease, and thus require proper control strategies to prevent this agent from spreading to other does. A monitoring program to culture milk samples on regular basis might be a tool to detect the infected animal and separate them from the healthy animal to reduce the prevalence of subclinical mastitis in the healthy animal.

In addition to *S. aureus*, we also have identified *S. epidermidis* and *Bacillus* spp. from milk samples that were positive for caprine subclinical mastitis in this study. In caprine mastitis, *S. epidermidis* intramammary infections are associated with subclinical mastitis where it is identified in 20-30% of cultured samples (Contreras *et al.*, 2003). *Bacillus* spp. has also been identified from the caprine subclinical mastitis quite frequently (El Idrissi *et al.*, 1994; Kostelić *et al.*, 2009). In the present study, two milk samples were positive for Gram-negative rod along with other organisms. The ability of Gram-negative bacteria to induce mastitis in goat has earlier been reported by Ryan and Greenwood (1990) and Contreras *et al*. (1995).

Present study was carried out on a small population in Mymensingh. Further and intensive epidemiological studies should be carried at regional and national level to determine the prevalence of caprine subclinical mastitis, identification of the risk factors and characterization of the associated bacterial agents for the treatment and control of the disease.

## Conclusion

The prevalence of subclinical mastitis in goats in Mymensingh area of Bangladesh was 18.64%. *S. aureus* was the most common bacterial species associated with subclinical mastitis in goats in this vicinity. Certain risk factors as well as some protective factors associated with subclinical mastitis were identified. Age of the animal, parity, length of lactation period and housing system influenced the prevalence of subclinical mastitis status in goat population studied. Present findings suggest that application of broad spectrum antibiotic could be an effective way to control the disease along with some modifications in the farm management system.

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