

Epidemiological studies on subclinical mastitis in dairy goats in northern regions of Bangladesh

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Abstract

A cross sectional study was carried out from June, 2008 to December, 2010 to estimate the prevalence of subclinical mastitis and to determine the responsible bacterial pathogens in lactating dairy goats in northern districts of Bangladesh. A total of 292 goats and 584 udder halves milk samples were screened using California Mastitis Test (CMT). Prevalence of subclinical mastitis at goat level was 56.2% (164/292), and in udder half level it was 33.9% (198/584). The subclinical mastitis prevalence at goat level was high (71.6%) in Jamnapari goats as compared to Black Bengal goats (50.2%), whereas at the udder half level, subclinical mastitis prevalence was 45.1% and 29.6% in Jamnapari and Black Bengal goats, respectively. The pathogens isolated from subclinical mastitic milk samples were coagulase negative *Staphylococci*, Coliforms, *Streptococcus spp.*, *Staphylococcus aureus*, *Micrococcus spp.*, *Pseudomonas aeruginosa* and *Bacillus cereus*. Among these, the most frequent isolates were coagulase negative *Staphylococci* (28.8%), Coliforms (22.7%) and *Streptococcus spp* (15.2%). Out of four potential host related risk factors considered, litter size and body condition of goats were found to influence the prevalence rate of subclinical mastitis in goat significantly ($p < 0.05$). On the other hand, the subclinical mastitis was very significantly ($p = 0.0001$) associated with the housing system of goats, i.e., goats reared in raised floor had a low subclinical mastitis infection rate (35.8%) as compared to reared in earth floor (62.2%). Antibioqram studies were also performed for the bacterial isolates and Gentamicin was found to be the most effective drug.

Key words: Antibioqram, California Mastitis Test (CMT), mastitis, prevalence, risk factors

Introduction

Mastitis is a persistent, inflammatory reaction of the udder tissue. It is the most common costly disease in dairy animals worldwide (Harmon, 1994). Dairy goats produce about 15.2 million metric tons (MT) of milk, accounting for about 2% of the world total amount of milk produced by livestock species, the largest amount of goat milk is produced in India, followed by Bangladesh and Sudan (FAOSTAT, 2008). The dairy goat industry is rapidly gaining in importance throughout the world in recent years. Among the several problems hindering the livestock development in Bangladesh, disease problems constitute a severe threat to the successful production of livestock and its industry. Therefore, any factor that adversely affects the quantity and quality of cattle and goat milk is of great financial interest. Milk quality is mainly affected by bacterial contamination of the mammary gland, which causes

clinical or subclinical mastitis (Boscos et al. 1996). Mastitis is defined as an inflammation of the mammary gland, affects lactating animals including cattle, goats, sheep, buffaloes and camels and is almost always associated with bacterial infection. Mastitis in the goat is mainly subclinical (Contreras et al. 1995 and McDougall et al 2002). It is one of the major problems of the dairy industry worldwide including Bangladesh. Subclinical mastitis is considered more severe than clinical mastitis, as early detection is impossible without regular monitoring. Subclinical mastitis is characterized by no visible sign of disease, apparently normal milk, with an increase in somatic cell count (SCC) that is bacteriologically positive (Blowey and Edmondson 1995). Subclinical mastitis results in decreased milk yield in goat in consequence growth retardation and higher mortality rate among suckling kids. The California Mastitis Test (CMT) developed by Schalm and Noorlander (1957) has been routinely used to evaluate the content of leucocytes and epithelial cells, defined as the somatic cells (SC), in milk. The CMT was also chosen in several investigations because it is more perfect, efficient and reliable than other field and chemical tests for diagnosis of subclinical mastitis (El-Balkemy et al. 1997). The primary causes of subclinical mastitis in goats are *Staphylococcus spp.*, *Streptococcus spp.* and *Micrococcus spp.* (Boscos et al. 1996; Ndegwa et al. 2001). All immunization procedures developed against mastitis have remained unsuccessful due to its multiple etiological agents. Antibiotic therapy is an important tool in the scheme of mastitis control. The treatments are more effective when directed by veterinarians; for example correct drug selection can be enhanced using an appropriate antimicrobial susceptibility test. The misuse or intensive use of antibiotics can lead to the development of resistance among different bacterial strains. Therefore, regular studies on antibiotic sensitivity of bacterial isolates are mandatory for effective and economical treatment of the disease (Sanchez et al. 1988). Although milk is a very nutritional food that is rich in carbohydrate, proteins, fats, vitamins and minerals but health risk to consumers can be associated with milk, due to the presence of zoonotic pathogens and antimicrobial drug residues (Bradely, 2002).

Due to the above mentioned economic and public health importance, this study was designed to estimate the prevalence of subclinical mastitis in apparently healthy dairy goats, to elucidate the associated risk factors and to find out the most frequently causative agents causing IMI, and to do antibiogram of the major bacterial pathogens from milk samples of mastitic goats.

Materials and Methods

Study area and period

Field survey for the prevalence of subclinical mastitis was done at all the northern districts (both Rajshahi and Rangpur division) of Bangladesh. All the laboratory investigations were conducted at the Molecular Biology Laboratory of Institute of Biological sciences, University of Rajshahi, Bangladesh. The study was conducted for the three years period of from June, 2008 to December, 2010.

Study design

This was a cross-sectional study whereby study animals were selected randomly and inclusion criteria were that all apparently healthy lactating dairy goats were eligible for the study.

Study population

The study was conducted on 292 dairy goats belonging to private and house hold goat farms in all districts of Rajshahi and Rangpur division of Bangladesh. Of all 292 dairy goats, Black Bengal goats and high yielding Jamnapari goats were included, as 211 Black Bengal goats and 81 Jamnapari goats.

Questionnaire based data collection

The data were collected in a questionnaire specifically designed for this purpose. The questionnaire was pre-tested prior to their final use. The questionnaire was administered to the participating farmers, who answered questions about farm management and animal husbandry practices including host and environmental factors influencing the incidence of mastitis.

Physical examination of milk sample

Immediately after collection, milk samples were subjected to physical examination with naked eyes to detect any abnormalities in color, odor, consistency, presence of blood and clot, flakes and any other visible abnormalities.

Collection of milk sample

Milk sample was collected from 198 udder halves of goat. Before collection of sample each teat was thoroughly disinfected with cotton swabs and 70% alcohol. Special attention was paid to the teat ends. Sterile sample tubes which had been color-coded per –udder half were used. After disinfection, a few ml of milk from each udder half were milked into the corresponding sample tubes. Care was taken so that no contact was made between the stream of milk and the milker's hands. The tubes were held as horizontally as possible to minimise the entry of dust. The sterile rubber stoppers were held in such a way that they would not be contaminated. After taking samples from each udder half, the goat identification number was marked on each tube with a water proof marker pen. The tubes were transported on ice box to

Molecular Biology Laboratory of the Institute of Biological Sciences, University of Rajshahi, where they were stored at -20°C until cultured on standard bacteriological media.

California Mastitis Test (CMT) kit

CMT kit (Leucocytest[®], Synbiotics Corporation-2, rue Alexander Fleming -69007 Lyon, France and marketed by Advance Chemical, Co. Bangladesh Ltd.) was used in this study (Plate 3.2). The Leucocytest reagent is composed of Alkyl Aryl sulfonate (3%), sodium hydroxide (1.5%) and bromocresol purple (1:10,000) as an indicator.

California Mastitis Test (CMT)

Subclinical mastitis was diagnosed based on CMT results and the nature of coagulation and viscosity of the mixture (milk and CMT reagent), which show the presence and severity of the infection, respectively (Harmon, 1994). Before sample collection for bacteriological examination, milk samples were examined for visible abnormalities and were screened by the CMT according to Quinn *et al.* (1999). From each udder half, a squirt of milk sample was placed in each of the cups on the CMT paddle and an equal amount of 3% CMT reagent was added to each cup and mixed well. According to the visible reaction of the CMT, the results were classified into four scores: Negative (no change in consistency), Trace (slightly positive), 1 (mild positive) 2 (Moderate positive) and 3 (highly positive). Scores depend on the degree of gellation that was indicated by gelatinous mass in proportion to severity of infection present (Schuppel and Schwoppe, 1998).

Bacteriological cultures

Each CMT positive milk sample was separated under aseptic conditions in labeled sterile screw capped bottles. All milk samples were sent directly to the laboratory with a minimum of delay for routine culture techniques. Milk samples were cultured onto 10% sheep blood agar and MacConkey agar plates. Suspected colonies were identified morphologically, microscopically and biochemically according to Hargital *et al.* (1992).

Antibiotic sensitivity test

Antibiotic susceptibility test was performed using the Kirby-Bauer disc diffusion method (Bauer *et al.* 1966). Disc diffusion test is based solely on the presence or absence of a zone of inhibition around the antibiotic containing discs. Disc diffusion test were performed and interpreted according to the recommendations of the National Committee for Clinical and Laboratory Standard (NCCLS, 2001) by using Oxide discs impregnated with specific antibiotics. Statistical analysis of the experimental data

Factors that usually affect the prevalence of subclinical mastitis were recorded and were analyzed by using SPSS (Statistical Package for the Social Sciences) version 17.0 software and statistically significant associations were determined by the chi-square test.

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Results

Prevalence of subclinical mastitis in goat

Among 584 udder halves milk of 292 goats were examined for the diagnosis of subclinical mastitis and in doe level subclinical mastitis was found to be 56.2%.

Prevalence of subclinical mastitis in different breeds of goat

In case of goat level in Black Bengal and Jamnapari goats subclinical mastitis prevalence was found to be 50.2% and 71.6% respectively using CMT. Where as in udder half level subclinical mastitis prevalence was found to be 29.6% in Black Bengal goats and was 45.1% in Jamnapari goats. The result revealed that subclinical mastitis prevalence was significantly ($p < 0.05$) influenced by the breed of goats both at doe and udder half levels.

Table 1: Prevalence of subclinical mastitis in different breeds of goat

Host	Goats Level			Udder halves Level			Chi-square Value	Sig. Level
	Total goats	infected goats	% infected	Total Udder halves	infected udder halves	% infected		
Black Bengal	211	106	50.2	422	125	29.6	11.19	**
Jamnapari	81	58	71.6	162	73	45.1		
Total	292	164	56.2	584	198	33.9		

**significant at 1% level ($p < 0.01$)

Risk factors of subclinical mastitis in goat

In the present study in case of goat out of the different potential risk factors, litter size, parity, stage of lactation and body condition in addition to the environmental factors were studied. These factors were considered in chi-square statistical analysis test to find out their effects on the frequency of subclinical mastitis infection of lactating goats.

Host factors influencing prevalence of sub-clinical mastitis

Out of four potential host related risk factors considered (Table 2 such as litter size and body condition of goats were found to be significantly ($p < 0.05$) influencing the prevalence rate of subclinical mastitis in goat.

Goats with three kids had a significantly low subclinical mastitis infection rate (41%) as compared to that with single kid (64.7%). The result also revealed that subclinical mastitis was significantly ($p = 0.0001$) influenced by the body condition of goats (Table 2). Goats of good body condition group had a significantly low subclinical mastitis (39.5%) as compared to poor body condition group (69.1%). Parity and stage of lactation were not significantly associated with prevalence of sub-clinical mastitis ($p > 0.05$).

Table 2: Prevalence of subclinical mastitis of goat in relation to host factors

Risk factors	Goats Level			Udder halves			Chi-Sq. Value	Sig. Level
	Total goats (292)	infected goats	% infected	Total Udder halves (584)	infected udder halves	% infected		
Litter size								
1	116	75	64.7	232	92	39.7	7.52	NS
2	137	73	53.3	274	88	32.1		
3	39	16	41	78	18	23.1		
Parity								
Few(≤ 3)	182	97	53.3	364	117	32.1	4.89	NS
Moderate(4-7)	82	46	56.1	164	58	35.4		
Many(≥ 7)	28	21	75	56	23	41.1		
Stage of lactation								
Early(≥ 1)	117	56	47.9	234	65	27.8	5.74	NS
Mid(2-3)	101	64	63.4	202	79	39.1		
Late(≥ 4)	74	44	59.5	148	54	36.9		
Body condition								
Good	124	49	39.5	248	64	25.8	24.61	**
Medium	29	19	65.5	58	20	34.5		
Poor	139	96	69.1	278	114	41		

**significant at 1% level ($p < 0.01$)

NS=Not significant

Environmental factors affecting subclinical mastitis infection in goat

Housing

The result of the present study revealed that subclinical mastitis was significantly ($p = 0.0001$) influenced by the housing system of goats (Table 3). Goats reared in raised floor had a significantly low subclinical mastitis infection rate (35.8%) as compared to reared in earthen floor (62.2%).

Season

The present study indicated that subclinical mastitis was not significantly ($p > 0.05$) influenced by the season (Table 3). Goats in rainy season had a subclinical mastitis infection rate 57.8% and in case of dry season subclinical mastitis infection rate was 32.9%.

Table 3: Prevalence of subclinical mastitis of goat in relation to environmental factors

Risk factors	Goats Level			Udder halves Level			Chi-Square Value	Sig. Level
	Total goats (292)	infected goats	% infected	Total Udder halves (584)	infected udder halves	% infected		
Housing								
Earthen	225	140	62.2	450	168	37.3	14.60	**
Raised	67	24	35.8	134	30	22.4		

Season								
Dry season	178	93	52.2	356	116	32.9	2.86	NS
Rainy season	114	71	62.3	228	132	57.8		

**significant at 1% level ($p < 0.01$)

NS=Not significant

Bacterial isolates of subclinical mastitis infected goat milk

Milk samples were collected from 198 udder halves of 164 lactating goats. Of all udder half samples, 186 (93.9%) samples were culture positive and 12 (6.1%) samples yield no bacterial growth. Isolates from positive cases were 57 (28.8%) coagulase negative *Staphylococci* (CNS), 45 (22.7%) Coliforms, 30 (15.2%) *Streptococcus spp.*, 19 (9.6%) *Staphylococcus aureus* (CPS), 14 (7.1%) *Micrococcus spp.*, 11 (5.6%) *Pseudomonas aeruginosa*, and 10 (5.1%) *Bacillus cereus*. Most of the subclinical cases were due to coagulase negative *Staphylococci* (CNS), Coliforms, *Streptococcus spp.*, and *Staphylococcus aureus* (CPS) (Table 4).

Table 4: Frequency distribution of the different bacterial isolates from the milk samples of subclinical mastitis positive goats

Serial no.	Bacterial species	No. of samples	Frequency(%)
1	Coagulase-negative <i>Staphylococci</i> (CNS)	57	28.8
2	<i>Staphylococcus aureus</i> (CPS)	19	9.6
3	<i>Micrococcus spp.</i>	14	7.1
4	<i>Streptococcus spp.</i>	30	15.2
5	Coliforms	45	22.7
6	<i>Pseudomonas aeruginosa</i>	11	5.6
7	<i>Bacillus cereus</i>	10	5.1
8	No Growth	12	6.1
	Total	198	100

Results of antibiotic sensitivity pattern of isolated bacteria

The antibiotic sensitivity patterns of bacterial isolates are presented in Table 5. In this trial, seven most commonly used antibiotics were used. It is evident from this table that the average sensitivity of the isolates was in descending order to gentamicin, ciprofloxacin, amoxicillin, ampicillin, erythromycin, tetracycline and penicillin. Gentamicin was found to be the most effective and penicillin the least effective against various mastitis pathogens in this study.

Table 5: *In vitro* antibiotic sensitivity results of the bacterial isolates

Bacterial isolate	% of sensitive isolates against						
	Gentami cin	Ciprofloxacin	Erythro mycin	Tetracyc line	Amoxici llin	Ampici llin	Penicillin
<i>Staphylococcus aureus</i>	93.3	93.3	86.6	20	80	73.3	66.6
Coagulase-negative <i>Staphylococci</i>	93.3	80	86.6	33.3	73.3	60	40
<i>Streptococcus spp.</i>	95	90	80	35	75	70	45

Coliforms	85	75	15	45	65	35	0
<i>Pseudomonas aeruginosa</i>	85	80	10	45	70	50	0
<i>Bacillus cereus</i>	90	80	15	55	65	45	0
<i>Proteus spp.</i>	85	70	20	45	60	35	0

Discussion

In the present study overall prevalence of subclinical mastitis was 56.2% by CMT in the goat population studied. This observed prevalence is close to the result obtained by Poutrel et al. (1997) & White and Hickley (1999)...what about their observations/values??. But the present report of doe-prevalence of subclinical mastitis is relatively higher than many other workers like Kostelic et al. (2009) recorded as 20% and Ndegwa et al. (2000) as 9.8%. This may be due to the influence of several factors such as breed differences, age and parity of the animals, stage of lactation and body condition, different management practices followed on each farm (Contreras et al. 1995; Boscos et al. 1996 and McDougall et al. 2002).

The present study indicated that prevalence of subclinical mastitis is significantly ($p < 0.05$) influenced by the litter size and body condition of goats, because goats with more kid and poor body condition are usually suffer from malnutrition and energy deficiency as a result easily become the host of infectious agents due to low immunity. Although there was no significant association between the prevalence of subclinical mastitis and stage of lactation, an obvious trend of increasing prevalence rate of subclinical mastitis with the stage of lactation was observed. This result is similar to the finding of East et al. (1986) who found no association between parity and subclinical mastitis status. Prevalence of caprine subclinical mastitis also appeared to be influenced by farm management system such as type of housing used to raise the goat in the studied area. The prevalence of subclinical mastitis was significantly higher in farms where goat was raised under traditional conditions and had an earth floor (soil surface). Earth floor has been reported to be an important risk factor for subclinical mastitis in goat (Ndegwa et al. 2000; Megersa et al. 2010). This observation could be explained by the fact that dirty and wet bedding is a common finding on the earthen floors, tends to harbor a wide range of infectious agents that may contaminate the udder and teats. On the basis of these findings, we can suggest to use semi-intensive farming system with either raised floor or concrete floor to rear goats in order to reduce the prevalence of subclinical mastitis.

In the present study, most of the subclinical mastitis infections were due to coagulase negative *Staphylococci* species accounting for about 28.8% of the infections. This finding is in agreement with the result of Leitner et al. (2001). Though coagulase negative *Staphylococci* are regarded as minor pathogens and normal inhabitants of mammary gland some studies indicated that coagulase negative *Staphylococci* could be pathogenic and even cause more mastitis than *Staphylococcus aureus*.

Other than coagulase negative *Staphylococci* bacteria, Coliforms, *Streptococcus spp.* and *Staphylococcus aureus* (CPS) were the second common pathogens observed in both cattle and

goat subclinical mastitis infection, considered as environmental pathogens. Higher incidence of environmental pathogens may be due to poor hygienic conditions; these environmental pathogens originate from the cow's environment and infect the udder via the teat canal (Mallikarjunaswamy and Karishina, 1997). These results regarding the distribution of pathogens correspond well with other studies on subclinical mastitis infection in dairy goats throughout the world (Ndegwa *et al.*, 2001; Leitner *et al.*, 2004), in which coagulase negative *Staphylococci* are the predominant organisms isolated from bacteriological positive milk samples. The large proportion of coagulase negative *Staphylococci* infections is consistent with the absence of clinical IMI, in fact, some authors have proposed coagulase negative *Staphylococci* as etiological agents of subclinical IMI in goats (Leitner *et al.*, 2004) and cows (Smith and Hagstad, 1986). In addition, *Staphylococcus aureus*, whose role in clinical mastitis in goats has been clearly defined (Maisi and Riipinen, 1991) and subclinical infections by *Staphylococcus aureus* are also common in goats (Moroni *et al.*, 2004). Finally, it may be said that in this study compared to environmental pathogens a significant number of isolates were contagious pathogens; appropriate milking practice such as milking of affected goats last, use of separate towels to wash and dry and milker's hand hygiene and culling could be feasible options in the control program in this region.

The *in vitro* antibiogram studies of the bacterial isolates from mastitis milk revealed gentamicin (90%) to be most effective drug followed by ciprofloxacin (83%), amoxicillin (71%), ampicillin (55%), erythromycin (49%), tetracycline (39%) and penicillin (25%).

Gentamicin, ciprofloxacin and erythromycin are newer chemotherapeutic agents and are less commonly used for the treatment of mastitis in the area of study resulting in higher efficacy of these drugs. Gentamicin proved to be the drug of choice in this study. Similar antibiogram pattern was reported by Frigerio *et al.* (1995). In this study, most of the bacterial isolates showed resistance to penicillin, and tetracycline. Indiscriminate and frequent use of these antibiotics in animals could be the reason for their ineffectiveness against bacterial isolates. Production of plasmids mediated beta-lactamase enzymes is supposed to be mainly responsible for resistance to penicillin. Since, streptomycin has been extensively used along with penicillin for treating mastitis; it may have led to the development of high resistance in bacteria against this antibiotic. Present results regarding tetracycline resistance for *Staphylococcus aureus* and coagulase negative *Staphylococci* were similar to those in Finland (Myllys *et al.*, 1998). A possible explanation for this phenomenon could be that for many years tetracycline has been the most widely antimicrobial class used by the farmers to treat any infection. On the other hand, less effectiveness of amoxicillin to all the isolates may be due to the resistance produced in the bacteria due to extensive use of this antibiotic in goats.

Conclusion

The prevalence of subclinical mastitis in goats in northern districts of Bangladesh was 56.2%. Coagulase negative *Staphylococci* were the most common bacterial species associated with subclinical mastitis in goats in these areas. Identified risk factors associated with subclinical mastitis were litter size, body condition and housing system. Present findings suggest that regular screening of animals for subclinical mastitis with some changes in the farm management system could be an effective way to control the clinical mastitis in dairy industry.

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