



RESEARCH ARTICLE

Identification and Antibiotic Sensitivity of the Causative Organisms of Sub-clinical Mastitis in Sheep and Goats

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ABSTRACT

This study was conducted to isolate and identify organisms responsible for sub-clinical mastitis (SCM) in goats and sheep for the determination of point prevalence of SCM and antibiotic sensitivity of the identified organisms. For this purpose 50 each of lactating sheep and goats were examined with the commercially available Leucocyttest[®] SCM detection kit. It was found that 4 and 36% sheep and goats suffered from SCM, respectively. The prevalence of clinical mastitis (CM) was 4 and 6% in sheep and goats, respectively. Milk samples were collected individually from sheep and goats with SCM and were cultured in different media including nutrient agar, blood agar and eosin methylene blue agar. The bacteria were further characterized by biochemical tests. In both goats and sheep, the organisms responsible for SCM were *Staphylococcus aureus* and *Escherichia coli*. The organisms were found most sensitive to gentamicin (Gn). After Gn treatment to goats and sheep with SCM, total bacterial counts decreased and milk production significantly increased compared to levels prior to treatment. It is suggested from the study that early detection of SCM and treatment with proper antibiotics can control SCM in goats and sheep.

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INTRODUCTION

Mastitis is an inflammatory condition of the mammary gland, characterized by changes in the physical characteristics of the udder or milk (Nazifi *et al.*, 2011). Mastitis can be classified into three major types: clinical mastitis (CM), sub-clinical mastitis (SCM) and chronic mastitis (ChM) (Anonymous, 2003). In CM, there are changes in milk color, clots are present in the milk and there are large numbers of leukocytes in the milk. Swelling, heat, pain, and indurations may be observed in the mammary gland in clinical cases; these symptoms can be detected by visual observation of the udder. In SCM, there are no clinical signs of disease other than an increased somatic cell count (SCC) in the milk, the presence of pathogenic organisms in the milk, and an inflammatory response that can only be detected by

screening or laboratory tests. ChM is an inflammatory process that has lasted for months and may continue from one lactation to another.

Obviously SCM is one of the most important infectious diseases in small ruminants. Furthermore, SCM represents a constant risk of infection for the whole stock. As there is a need for higher milk yields and more stringent requirements on milk quality in dairy goat herds, udder infections must be prevented or detected at an early stage not only to protect the farmer but rather the consumer.

The most important bacterial species responsible for mastitis is *S. aureus*, and its prevalence in dairy herds varied widely from 7 to 40% (Fox and Gay, 1993). Byeng *et al.* (2007) reported that coagulase negative *Staphylococcus* (43.7%), *S. aureus* (35.4%), and *Pseudomonas aeruginosa* (12.4%) were the most prevalent pathogens in SCM of goats.

Mastitis is diagnosed based on clinical signs, an increase in the SCC and identification of bacteria in the milk (Droke *et al.*, 1993). In dairy animals, the SCC is directly correlated to the severity of the mastitis (Pantoja *et al.*, 2009). Factors like season, milk fever prevalence and hygiene sanitary management conditions may affect the SCC (Koop *et al.*, 2009). The SCCs are higher in goats than cattle which could be due to higher quantities of cytoplasmic particles and epithelial cells in goat milk (Paape and Capuco, 1997). Therefore, species differences should be taken into account when interpreting an increased SCC.

Mastitis can be cured by treatment with antibiotics after identification of the causative agents. Antibiotic sensitivity tests can be performed to ensure adequate treatment. In sheep, intramammary antibiotic therapy using a combination of penicillin, nafcillin, and dihydrostreptomycin has been found to be effective in reducing the load of mastitis pathogens after lambing (Chaffer *et al.*, 2003).

Mastitis has significantly constrained the development of the dairy industry in Bangladesh. Reduction in milk production due to mastitis is responsible for serious economic loss to the dairy industry; furthermore, animals with mastitis act as carriers of mastitis-inducing bacteria and source of infection for healthy animals (Tanja and Karen, 2010). The present study was undertaken to determine the point prevalence of SCM in sheep and goats in Bangladesh, to identify the causative organisms, and to determine their antibiotic sensitivities.

MATERIALS AND METHODS

Fifty each of lactating Bengal sheep and Black Bengal goats were randomly selected from goat and sheep farm of Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, Bangladesh, and tested for both CM and SCM. The animals were reared under natural conditions, and a uniform and constant nutritional regime was maintained.

Assessment of clinical and sub-clinical mastitis:

Clinical mastitis was assessed by palpation and visualization of the udder, and diagnosed if the udder was red, hard, or hot to touch. Mild CM was judged visually by slight or moderate swelling, indurations of one or more quarters, and a visibly abnormal secretion, including clots, revealed by the use of a strip-cup. In severe CM, the udder was swollen and milk secretion was grossly abnormal. Animals sometimes had an increased body temperature, loss of appetite, depression, and nearly complete cessation of milk secretion.

Mastitis was suspected when the teats showed any lesions including eruptions, swelling, teat blockages, abscesses, or flakes. On the basis of the degree of infection, CM was assessed by a strip-cup test. Briefly, milk was drawn from the teats of sheep or goats onto a piece of black screen plate and after 10 to 15 seconds, the presence of any fibrous flakes, wateriness, or bloodstains was considered as indication of mastitis.

Sub-clinical mastitis was assessed by the California mastitis test (CMT) using 10% Teepol (Leucocyttest®; Synbiotics Corporation, France). CMT was done

according to manufacturer's instructions. Briefly, in each well of the test plate, 2 ml of milk was stripped from individual teats and an equal amount (2 ml) of 10% Teepol was added to the milk. A circular motion was made with the plate for 10 seconds to mix the reagent and milk, and after 20 seconds, changes in the milk were observed. The formation of milk clots upon addition of the reagent was recorded.

Bacterial isolation and antibiotic sensitivity: Milk samples from animals with SCM were collected aseptically. The collected milk samples were cultured on different agar media, namely nutrient agar (NA), blood agar (BA), and eosin methylene blue (EMB) agar. The organisms were identified on the basis of colony morphology, Gram staining results, characteristic hemolytic patterns, and biochemical tests (Balows *et al.*, 1991). Gram staining was performed as described by Murray *et al.* (1994). The color and morphology of the bacteria were observed by microscopy.

Each sample was sub-cultured on NA, BA, and EMB agar plates. Streptomycin (S), penicillin (P), gentamicin (Gn), ampicillin (Amp), amoxicillin (Amx), neomycin (N), and oxytetracycline (OT) antibiotic discs were placed on the culture media containing the bacterial samples, and plates were incubated at 37°C for 24 hours.

Animal treatment: The SCM-infected animals were treated with the antibiotic to which the bacteria cultured from the milk were most sensitive based on antibiotic sensitivity tests. Animals were treated with antibiotic i.m. for 3 days. The total bacterial count (TBC) of the milk samples from sheep and goats was estimated before and after antibiotic treatment. TBC was accomplished as per Dahal *et al.* (2010). Milk samples were cultured on NA plates. Milk production was recorded on days 0, 7, and 15 after the start of antibiotic treatment. Data obtained from TBC and milk production were analyzed with Student's *t*-test and $P < 0.05$ was considered as significance level.

RESULTS

Goats affected at high level by SCM compared to sheep (Fig. 1), while both goats and sheep were affected at low level by CM.

From the milk samples of goats, both Gram (+ve) and (-ve) bacteria were grown on the NA (Table 1), which were cocci and rod-shaped, respectively. Small and large, spread-out, whitish, round colonies were observed in the NA. Gram (-ve) rod-shaped bacteria were grown in EMB with light, shiny colonies. In the BA cultures of SCM-affected goat's milk, Gram (+ve) cocci bacteria were found. Pinpoint- to medium-sized, whitish colonies were grown on BA in addition to hemolytic characteristics. Similar observations were recorded in case of sheep milk, cultured on different media. *S. aureus* was identified from the milk samples of goats and sheep by confirming acid production in the fermentation of dextrose, lactose, sucrose, and mannitol. The identity of *S. aureus* was further confirmed based on a positive coagulase test. On the other hand, *E. coli* was confirmed by the fermentation of dextrose, maltose, lactose, sucrose, and mannitol, and a positive methyl red test.

Table 1: Gram-staining results of the bacteria from milk samples (n=18) of SCM-affected goats grown on nutrient agar, blood agar, and EMB agar.

Growth media	No growth %	% Gram (+ve) bacteria	% Gram (-ve) bacteria	% Gram (+ve) and (-ve) bacteria
Nutrient agar	0	50	11.11	38.88
EMB agar	61.11	0	38.88	0
Blood agar	38.88	61.11	0	0

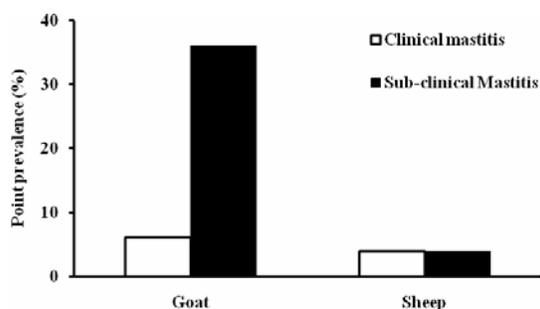


Fig. 1: Point prevalence of clinical and sub-clinical mastitis in goats and sheep at the BLRI farm. Clinical and sub-clinical mastitis were tested by the strip-cup test and California mastitis test, respectively, for 50 milking goats and 50 milking sheep.

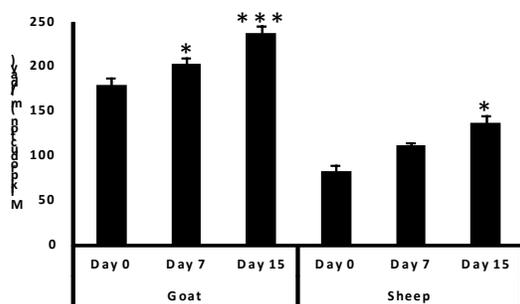


Fig. 2: Total bacterial count in milk samples of SCM-affected goats and sheep at day 0 (before treatment), 7 and 15 (after treatment). ***P<0.005.

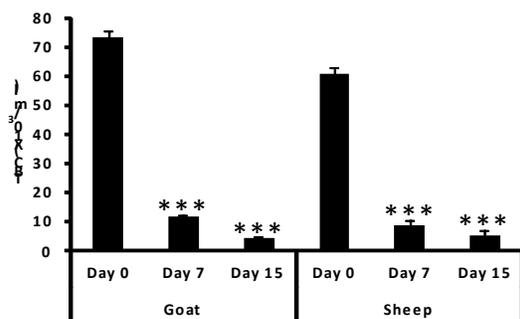


Fig 3: The average milk production of SCM-affected goats and sheep was measured at day 0 (before treatment), day 7, and day 15 (post-treatment). *P<0.05; ***P<0.005.

Of the antibiotics tested, the organisms that were identified in sheep and goat milk were overall the most sensitive to Gn. Bacteria from goat milk samples cultured on NA were most sensitive to Gn, followed by P and S, those cultured on EMB agar were most sensitive to Amp and Amx following Gn, and those cultured on BA were the most sensitive to Gn, then P and Amp. The bacteria in

sheep milk samples were most sensitive to Gn, followed by S and N (Table 2).

The TBC decreased remarkably between the first day of treatment and day 7 post-treatment; thereafter, it continued to decrease further until day 15 post-treatment (Fig. 2). At day 0, the average milk production in goats and sheep was 180 and 82.5 ml/day, respectively, but by day 15, the average milk production increased to 238.1 and 137.5 ml/day for goats and sheep, respectively (Fig. 3). These results indicated that treating animals with SCM with Gn reduces SCM shedding of the associated bacteria, resulting in an increase in milk production.

DISCUSSION

In this study, we found that 4% of sheep and 36% of goats infected with SCM were CMT-positive. The prevalence of SCM in ewes in southern Greece was reported to be 4.5% after weaning of the lambs (Fthenakis, 1994). McDougall *et al.* (2002) reported a prevalence of SCM 35.5% in goats and 19.0% in sheep, at parturition. Contreras *et al.* (2007) noticed a prevalence of SCM in goats of 5 to 30% after summarizing the results from various researches. In this study, *S. aureus* and *E. coli* were identified as causative bacteria of SCM in the milk samples of goats and sheep. Fthenakis (1994) also identified these bacteria as the causative organisms of SCM in ewes along with *S. epidermidis*, *S. simulans*, *S. chromogenes*, *S. xylosus*, streptococci, *Bacillus* spp., *Pasteurella haemolytica*, and *Actinomyces pyogenes*. *S. aureus* is the most significant pathogen of the caprine mammary gland (Contreras *et al.*, 2003). According to studies of Winter (2009) for the years 1998 to 2003, *S. aureus* can be detected in between 20.8% and 46.6% as a pathogen of SCM. Infections with *S. aureus* can be subclinical, chronic, acute, or gangrenous in their most severe form (White *et al.*, 1999). Infected animals do serve as a reservoir for further infection within the herd, and may shed the organism in milk intermittently. *S. aureus* is an important foodborne pathogen (Jorgensen, 2005) and is capable of producing several toxins (Fagundes *et al.*, 2010). Contaminated milk obtained from cows affected by subclinical mastitis is a potential source of staphylococcal food poisoning to consumers (Zecconi and Hahn, 2000).

Several studies have shown that Gram-negative bacteria rarely induce mastitis in goats (Ryan and Greenwood, 1990; Contreras *et al.*, 1995). In the present study, *E. coli* were isolated from the infected milk samples of both sheep and goats. Infections with *E. coli* usually lead to systemic illness and the secretion of abnormal milk that may contain blood, flakes, clots, and excess water. *E. coli* is an environmental pathogen, but maintaining a clean environment, milking clean, ensuring the teats are dry, and avoiding teat end injuries may reduce the prevalence of infection (Smith and Sherman, 1994).

Because most udder inflammation is sub-clinical, it is easy for farmers to neglect the disease. As a result, the infection can persist in the udder and SCM progresses to CM, which adversely affects the profits of dairy farms. The majority of dairy farmers in developed countries have introduced dry cow antibiotic therapy to control mastitis in their herds, which in many cases has proven to be cost-

Table 2: Antibiotic sensitivities of caprine and ovine sub-clinical mastitis pathogens. The percentage sensitivity (%) of the bacteria in 18 milk samples from SCM-affected goats and 2 milk samples of SCM-affected sheep to the antibiotics was calculated according to the highest zone of inhibition in different culture media.

Antibiotics	Goat milk samples (n=18)						Sheep milk samples (n=2)					
	NA		EMBA		BA		NA		EMBA		BA	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Gentamycin	17	94.44	16	88.88	18	100	2	100	2	100	2	100
Streptomycin	9	50	6	33.33	2	11.11	1	50	2	100	1	50
Ampicillin	6	33.33	10	55.55	10	55.55	-	-	-	-	-	-
Amoxicillin	5	27.77	8	44.44	6	33.33	-	-	-	-	-	-
Penicillin	12	66.66	0	0	15	83.33	2	100	1	50	0	0
Neomycin	-	-	-	-	-	-	2	100	1	50	0	0
Oxytetracycline	-	-	-	-	-	-	1	50	1	50	1	50

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Conclusions: We identified *S. aureus* and *E. coli* as the etiologic agents of SCM in goats and sheep of BLRI goat and sheep farm. Bacteria were most sensitive to Gn, and were also sensitive to the antibiotics P, S, Amp, Amx, and N. Milk production was increased noticeably after using Gn as a treatment against SCM organisms.

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