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Original Article

Prevalence and identification of subclinical mastitis in cows at BLRI Regional Station, Sirajganj, Bangladesh

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ABSTRACT

Objective: The study was conducted to assess the prevalence of subclinical mastitis in dairy cows at Baghabari milk shed area of Sirajganj and Pabna districts in Bangladesh.

Materials and methods: A total of 300 milk samples were collected from crossbred dairy cows, and the milk samples were subjected for using California Mastitis Test (CMT). Besides, data related to farm management were taken from 60 farmers through direct interviews. The CMT was compared with White Slide Test (WST) and Surf Field Mastitis Test (SFMT). The samples showing strong positive reaction to mastitis by CMT were used for the isolation of associated bacteria using conventional bacteriological examinations and biochemical properties. The isolated bacteria were subjected for antibiogram studies by disc diffusion method.

Results: Out of 300 samples, 153 (51%) revealed positive reactions denoting that the cows were suffering from subclinical mastitis. Of the 153 samples, 39(13%) were trace-positive, 56(18.56%) were weak-positive, 33(11%) were distinct-positive, and the rest 25(8.3%) samples were strongly positive. In comparison economically among CMT, WST and SFMT, SFMT was found to be the cheapest and easiest. In this study, the cows were mostly infected with mixed infection with *Staphylococcus spp.*, *Streptococcus spp.*, *Escherichia coli, Salmonella spp.*, and some cows had single bacterial infection. It was observed that most of the microorganisms were sensitive to Gentamycin, Amoxicillin and Ceftriaxone, and were resistant to Penicillin, Ciprofloxacin and Colistin sulphate.

Conclusion: Overall prevalence of subclinical mastitis in the crossbred dairy cows at Sirajganj and Pabna district is 51%. It indicates that subclinical mastitis is a major threat for dairy industry in the studied areas. Early detection and appropriate preventive measures are suggestive to successful control of the disease.

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KEYWORDS

Bacteria; Cows; Sub-clinical mastitis; Prevalence

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INTRODUCTION

Mastitis is characterized by increased somatic cell count in the milk of affected animal. Subclinical mastitis shows no changes in the milk apparently. However, production of milk can be reduced up to 80% due to subclinical mastitis. If the disease persists for long time it adversely affects milk quality (Seegers et al., 2003). The prevalence of mastitis is mostly found in the farm condition with larger hard size as compared to those of lower herd sizes (Radostits et al., 2000). Mastitis may originate from microbial cause; the microbes may include virus, bacteria, mycoplasma and yeast (Egwu et al., 1994).

Although difficult as compared to clinical mastitis, early detection of subclinical mastitis in cow is crucial for saving dairy cattle and reduction of losses of farmers. Considering physical and chemical changes of milk and isolation of associated organisms, several methods have been developed to identify subclinical mastitis (Badiuzzaman et al., 2015). Among the tests, California Mastitis Test (CMT), White Side Test (WST) and Surf Field Mastitis Test (SFMT) are arguably the reliable screening tests for sub clinical mastitis (Greiner et al., 2000).

Staphylococci are the major etiological agents followed by *Streptococci* and *Escherichia coli* causing sub-clinical mastitis in cows in India (Singh and Baxi, 1982). In Bangladesh, clinical cases of mastitis caused by several bacteria like *Staphylococci*, *Streptococci*, *Corynebacterium*, *E. coli* and *Bacillus sp.* (Mahbub-E-Elahi et al., 1996). Subclinical mastitis is a serious problem for dairy industry as there are no gross changes found in udder or glandular tissues. Thus, it needs laboratory examination. Sub-clinically infected animal may act as a continuous source of infection to herd mates. If sub-clinical mastitis persists in dairy industry, it causes huge losses. The annual economic loss due to reduced milk production in Bangladesh estimated to be Taka 122.6 (US$ 2.11) million (Kader et al., 2003). Considering the above situation, prevalence study of subclinical mastitis and identifying the causal agents are crucial. The objectives of the present study were to assess the prevalence of sub-clinical mastitis in cows in Sirajganj and Pabna districts of Bangladesh, to identify the causal agents, and to build awareness among the farmers.

MATERIALS AND METHODS

Selection of study area, duration and study animal: A total of 300 crossbred dairy cows were selected from 13 villages for this study and were sampled during the period of September 2015 to March 2016 from different dairy farms at Baghabari milk shed area of Sirajganj and Pabna districts in Bangladesh. Number of cows per farm was between 3 and 50. In this study, only apparently healthy crossbred dairy cows were considered.

Sample collection: In this study, 300 samples were collected from Baghabari milk shed area of Sirajganj and Pabna districts. Before collection of milk, the teat and tips were washed with clean water, antisepsis was done with a swab soaked with 70% alcohol and then milk samples were collected aseptically from the udder during morning. All the milk samples were collected in vials which were labeled with identification number of cow.

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

Detection of sub-clinical mastitis by CMT tester: For detection of sub-clinical mastitis, CMT was performed as the instructions of manufacturer (CHEIL BIO Co. Ltd.). In brief, 2 mL milk and 2 mL CMT solution were mixed together in test paddle. Rotate the paddle to mix, and changes in color and gel formation was observed within 10 to 15 Sec.

Transportation of milk sample: The milk samples of strong positive results (+++) in CMT were transferred to Animal Health Laboratory, Bangladesh Livestock Research Institute (BLRI), Regional Station Baghabari, Sirajganj and also to Animal Health Division, BLRI, Head Quarter, Savar, Dhaka by cool box with ice.

Isolation of bacteria from milk: Before incubation, the sample was allowed in normal temperature. Then 100 µL of milk sample was taken with micro pipette and the sample was expelled on a test-tube containing 10 mL nutrient broth. These works were performed within a biohazard safety cabinet for aseptic measure. Then the milk sample containing broth was incubated at 37°C for 24 h. After incubation, one loop of incubated sample was streaked on EMB agar, Mac Conkey agar, Brilliant Green Agar and Mannitol Salt Agar respectively and again incubated at 37°C for 24-48 h. The bacterial isolates were identified by their cultural, morphological and biochemical characters (Rahman et al., 2014). The classification and specification of organism was based on the scheme presented in Bergey's Manual of Systemic Bacteriology (Halt et al., 1985).

Antibiogram studies: The isolated bacterial were subjected for antibiogram studies using commonly used antibiotics (Gentamycin 10 µg/disc, Amoxicillin 20 µg/disc, Ceftriaxone 30 µg/disc, Penicillin G 10

units/disc, Ciprofloxacin 5 µg/disc and Colistin sulphate 10 µg/disc) following disc diffusion method, as described by Bauer et al. (1966).

RESULTS AND DISCUSSION

In this study, we found that farmers were practiced their animal deworming programs in different ways such as every 3, 6 and 9 months, 1 year and >1 year, and the percentage of it practicing farmers were 15, 50, 3.33, 26.67 and 5%, respectively. About 98 % farmers were not practicing of milking hand and udder washed out with antiseptic solution or water founded in this study. About 93 % farmers were not practicing of bathing of their dairy cows before milking and there were no certain hygienic place of milking. In this study, we found that 98% cow's sheds were tin shed and 76.67% floors were made up of brick. Hygienic status of housing and floor was not satisfactory for these dairy farms. Only 6.67% farmers used antiseptic in the cleaning program of their farms. They have no isolation shed for sick animals in their farms. Only 3.34% farmers adopted manure management system such as Biogas plant.

We found in this study that 54% dairy farmers claimed 30-60% reduced milk production due to mastitis in the affected cows. About 56.67% dairy farmers had previous record of culling of cows due to mastitis. Among the 300 cows, CMT was positive for 51% (trace- 13%, weak 11%, distinct 18.67%, strong 8.33%) animals. Among them, 100 were again screened with WST and SFMT showing negative 45 and 45%, mild 22 and 20%, moderate 27 and 29%, strong 6 and 6%, respectively. There are still factors related to the sanitary management that may directly influence on SCC (Ítavo et al., 2001).

Kader et al. (2002) reported similar report who described the prevalence as 44.61% sub-clinical mastitis in Bangladesh. On the other hand, higher prevalence (54%) of sub-clinical mastitis was recorded in India by Singh and Baxi (1982). As compared with early lactation (41.3%), prevalence of SCM was higher in late lactation period (58.5%), as reported by Radostits et al. (2000). Prevalence of sub-clinical mastitis was higher in high milk yielding animals (73%) as compared to low milk yielding animals (7.7%) (Khanal and Pandit, 2013). Previously, in Bangladesh, prevalence of sub-clinical mastitis in dairy cows was recorded as 72.07, 66.67, 64.86 and 61.26% by CMT, SCC, WST and SFMT, respectively, as reported by Badiuzzaman et al. (2015). The highest prevalence was found for CMT (67%) and WST (62%) in the animals aging 3.5-4.5 years (Khokon et al., 2017).

The result of this study was much higher than that of (Rahman et al., 2014) who reported only 20.13% in Friesian and more or less similar in case of Red Chittagong cows which was 13.24%. From India, (Devi et al., 1997) had reported 75.3% subclinical mastitis. This was also higher result than the present findings. Sargeant et al. (2001) studied the sensitivity of SCC and CMT for identifying intramammary infection during early lactation and found that CMT would better as compared to SCC. Barbosa et al. (2002) described that the SCC and CMT were dependent. Sudhan et al. (2005) reported that the percentage of agreement of CMT, SLST, bromothymol blue card test and WST with bacteriological examination

Table 1: Description of milk quality

Reaction	Decision	Somatic cell counts/mL	Polymorph nuclearleukocyte (%)
Mixture remain liquid state	-	0 ~ 200,000	0~25
A slight slime formed , Trace reactions tend to disappear with continued movement of the fluid	±	150,000~500,000	30~40
A distinct slime but with no tendency toward gel formation.	+	400,000 ~1,500,000	40~60
The mixture thickens with gel formation, collected to center of cups when rotate the test paddle.	++	800,000~ over 5,000,000	60~70
The mixture thickens with gel formation, icky adhere to base of cups. There is a central peak which remains projecting above the main mass after then motion of the paddle has been stopped. Viscosity is greatly increased so that there is a tendency for the mass to adhere to the bottom of the cup.	+++	Over 5,000,000	70~80
Indicated by a contrasting deeper purple color. Is's the result of inflammation or in drying-off of the mammary gland.	Alkali milk	Over 5,000,000	Over 80
Bromocresol purple is distinctly changes yellow color at pH 5.2	Acidic milk	Over 5,000,000	Over 80

Table 2: Cultural colony characteristics and Gram's staining reaction of the organisms isolated from sub-clinical mastitis affected cows.

MCA	XLD	MSA	EMB	Gram stain	Arrangement	Tentative diagnosis
No growth	No growth	Medium-sized yellow colonies	No growth	+ve	Cluster	*Staphylococcus spp.*
Rose pink lactose fermented colony	No growth	No growth	Yellow green metalic sheen	-ve	Single paired or in short chain	*Escherichia coli*
No growth	Black colony	No growth	No growth	-ve	Black spotted	*Salmonella spp.*

MCA=Mac Conkey Agar, XLD=Xylose Lysine Deoxycholate Agar, MSA=Mannitol Salt Agar, EMB=Eosin Methylene Blue agar

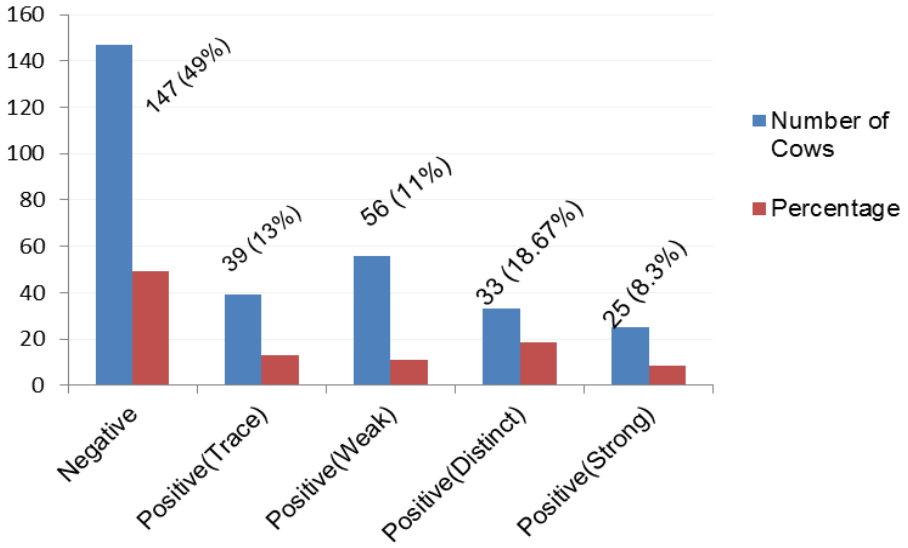

Figure 1. Prevalence of subclinical mastitis in dairy cows by CMT. Overall prevalence is 51%.

were 57.89, 62.07, 64.46 and 68.65%, respectively. This variation might be due difference in climatic condition, breed, management practices and treatment of cattle.

In this study, culture was done from the milk samples that demonstrated strong positive reaction in CMT. Mahbub-E-Elahi et al. (1996) isolated *Staphylococcus*, *Streptococcus*, *E. coli* and *Bacillus*, and Rahman et al. (1968) isolated and identified different strains of *Staphylococci* from mastitic and apparently healthy mammary glands of cows. These findings also corresponded with Shrestha and Bindari (2012) who reported highest prevalence of *Staphylococcus* followed by *E. coli*, *Streptococci* and *Corynebaceterium*. Chanda et al. (1998) reported that *Staphylococcus* was the principal organism of mastitis. *Staphylococcus* is the opportunistic bacterium which can survive the skin of the udder can infect via teat canal. In addition to *Staphylococcus*, another bacteria *E. coli* was identified in this study, which is an environmental opportunistic pathogen. Similar report was also reported by Mahbub-E-Elahi et al. (1996).

The occurrence of mastitis mainly depends on udder resistance, balance of pathogenic organisms and also period of the exposure of the lactic gland to infection (Janzekovic et al., 2009).

The isolates of *Staphylococcus spp.* were found to ferment all the five basic sugars and produced only acid whereas the isolates of *E. coli* were found to ferment all the five basic sugars and produced both acid and gas. Culture sensitivity test was performed against Gentamycin, Amoxicillin, Ceftriaxone, Penicillin, Ciprofloxacin and Colistin sulphate. It was observed that most of the microorganisms were sensitive to Gentamycin, Amoxicillin, Ceftriaxone and showed resistant to Penicillin, Ciprofloxacin and Colistin sulphate.

CONCLUSION

The prevalence of subclinical mastitis indicate that it is the major threat for dairy industry Further study should be taken into consideration to identify the specific causal agents and develop a mastitis detection kit or mastitis control vaccine for better prevention of the disease. Ensure early detection and taken preventive measures immediately then it will be helpful to control the disease successfully.

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CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

AUTHORS' CONTRIBUTION

HK, E, G and RI implemented the study design. MSI and YU participated in data collection and HK, also performed all the tests. HK and HR drafted, RK revised the manuscript. KHMNH critically checked the article and corrected the manuscript. All authors read and approved the final version of manuscript.

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