Wayamba Journal of Animal Science – ISSN: 2012-578X; P1706 - P1715, 2018
First Submitted July 08, 2018; Number 1525837904

PREVALENCE OF SUBCLINICAL MASTITIS AND ITS MICROORGANISMS IN LACTATING COWS AT SATKHIRA DISTRICT OF BANGLADESH

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This research was committed to evaluate the prevalence of bovine subclinical mastitis for isolation and identification of the microbial agents and predisposing factors associated with subclinical mastitis in randomly selected 250 lactating cows (179 local cattle and 71 (crossbreds) at Satkhira District of Bangladesh. California Mastitis Test was carried out each quarter sample of lactating dairy cows for evaluation of subclinical mastitis. Among 250 lactating cows, 66 (26.4%) were found to be positive for subclinical mastitis by California Mastitis Test. Out of 66 California Mastitis Test positive cows, only 19 cows showed strong positive reactions and these samples were used in culture media for isolation and identification of microbial agents. The prevalence of subclinical mastitis was significant in crossbreds (35.21%) as compared to local cattle (*Bos indicus*) (21.79%) . There was a dramatic shot up of prevalence in cows with a daily milk yield of 5-7 L and 7-10 L. The maximal prevalence found in mid-lactation was 30.99% and the greatest prevalence in the age group of 9 to 12 years was 37.5%. The prevalence of subclinical mastitis was comparatively superior (32.69%) in lactating cows were housed with fictile floor than that of the slatted floor (16.66%). Among the microorganisms isolated from the strongly positive subclinical mastitis samples, the most frequent microbes were *Staphylococcus sp*. (73.68%). For early detection of subclinical mastitis, California Mastitis Test can be accomplished regularly as a control measure and emphasis should be demonstrated on farm management practices, especially on milking equipments and hygienic udder.

Keywords: Microorganisms, California Mastitis Test, Prevalence, Subclinical Mastitis.

Bovine mastitis, an inflammation of the mammary gland, causes physical, chemical, and usually, bacteriological changes in milk and pathological changes in the glandular tissues of the udder that affect the quality and quantity of milk (Sharma and jeong., 2013). It has a high incidence and prevalence in dairy cows, affecting the net earnings of milk producers worldwide (Frola et *al*., 2011). Generally, clinical mastitis is easily diagnosed by visible clinical manifestations, such as red, hot, and swollen mammary glands (Sharma et *al*., 2007). Subclinical mastitis has no visible clinical symptoms in mammary glands and in milk, but milk production decreases, somatic cell count (SCC) increases, pathogens are present in the secretion, and the milk composition is altered (Lafarge et *al*., 2004). The subclinical form of mastitis in dairy cows is important because (a) this form is 15 to 40 times more prevalent than the clinical form (b) it usually precedes the clinical form (c) it is long duration (d) it is difficult to detect (e) it reduces production (f) it adversely affects milk quality (Seegers et *al*., 2003). A threshold of 200,000 cells/mL is indicative of subclinical mastitis (Dervishi et *al*., 2016). Moreover, the bovine teat surface can contain a high diversity of bacteria (Verdiermetz et *al*. 2012). Generally, *S. aureus, S. uberis, and S. agalactiae* are recognized as major virulent mastitis-causing bacteria (Kuehn et *al*., 2013) At least, 137 infectious causes of bovine mastitis are known to date, and in large animals the commonest pathogens are

Staphylococcus aureus, Streptococcus agalactiae, other Streptococcus species and Coliforms. It may also be associated with many other organisms including *Actinomyces pyogenes, Pseudomonas aeroginosa, Nocardia asteroides, Clostridium perfringens* and others like *Mycobacterium, Mycoplasma, Pasteurella* and *Prototheca* species, and yeasts (Radostits et *al*. 2007). Non-Aureus Staphylococci (NAS) are the bacteria frequently isolated from ud quarters in all recent North American and European Subclinical mastitis surveys. (De Vliegher et *al*., 2012; Dufour et *al*., 2012 ; Pyorala and Taponen.,2009; Sampimon et *al*., 2009a; Thorberg et *al*., 2009 ; Piepers et *al*., 2007). Intra-mammary Infection (IMI) with *Staphylococcus chromogens, Staphylococcus simulans, and Staphylococcus xylosus* have a greater effect on SCC compared with IMI with other species, such as *Staphylococcus cohnii* and *Staphylococcus sciuri* (De Visscher et *al*., 2016; Fry et *al*., 2014; Supré et *al*., 2011; Taponen et *al.*, 2007;). Mastitis is mainly categorized into clinical mastitis and subclinical mastitis (Kader et *al*., 2003). About 75-80% mastitis is subclinical, characterized by a significantly increased leukocyte count in milk.

It is now a well-known fact that the subclinical mastitis (SCM) is more serious and is responsible for much greater loss to the dairy industry (Kader et *al*., 2002). Khair (2006) reported that the prevalence of subclinical mastitis is 54% in Sylhet. The annual economic loss occurs due to reduced milk production alone caused by subclinical mastitis in Bangladesh has been estimated to be BDT 122.6 (US $ 2.11) million (Kader et *al*. 2003). Comprehensive reports on subclinical mastitis in Bangladesh are lacking, but a prevalence of 47% was recorded (Kader et *al*. 2003), and 55% was recorded in Sahiwal cows (Ghosh et *al*. 2004). Besides causing heavy economic losses to milk production, the subclinical mastitis remains a consecutive source of infection to other herds, as there are no significant inflammatory changes in the udder tissue, it is generally not detected until laboratory examination. If the infection persists for longer times, then it may lead to fibrous tissue barrier between the organisms and the prepared antibiotic. Furthermore, it is necessary to learn the prevalence of subclinical mastitis in dairy herds and illustrates the important factors responsible for it. California Mastitis Test (CMT) has been recognized as a highly sensitive test to detect bovine subclinical mastitis (Dangore *et al*. 2000, Sachin & Suresh 2006, Madut *et al*. 2009). So far, there is no report on prevalence of subclinical mastitis in dairy cows at Satkhira district of Bangladesh. Therefore, the study was designed to determine the prevalence and risk factors of subclinical mastitis for isolation and identification of microorganisms in lactating cows at Satkhira district of Bangladesh.

MATERIALS AND METHODS

Study area and study population

A study was carried out randomly selected 250 lactating cows (179 local zebu cattle (*Bos indicus*) and 71 (crossbreds) of dairy cattle containing at Satkhira district of Bangladesh during the period of 1st April 2017 to 31st March 2018. Dairy cows from large, medium and backyard farm are the source of population. The errand on animals like age, breed, parity, stage of lactation, daily milk yield, and type of floor used for housing of the animals was recorded using structured questionnaire by collecting data from the owners and/or attendants of the animals. Udder and milk abnormalities (injuries, tick infestations and indurations, swelling, milk clots, abnormal secretions etc. were recorded. The data was also collected from hospital record book.

Aseptic milk sample collection and physical examination

Milk sampling was carried out following aseptic procedures as described by National Mastitis Council (NMC, 2004). Approximately 10 ml aseptic fresh milk samples were collected from each quarter of randomly selected apparently healthy cows in separate glass tube according to the method described by Rosenberger (1979) at morning milking and the tube was labeled with the number of the cow. Immediately after collection, milk samples were subjected

to physical examination with naked eyes to track out any abnormalities in color, odor, consistency, and presence of the clot, blood, flakes and any other visible abnormalities. These aseptically collected milk samples were carefully used for CMT. The California mastitis test (CMT) was used as a screening test for sub-clinical mastitis. Microorganisms in raw milk directly impact the
flavor, quality, and shelf life of milk products. Moreover, the presence of pathogens in raw milk can lead to severe illness (Quigley et al., 2013; Oliver et al 2009).

Determination of subclinical mastitis

The number of two hundred lactating cows were tested with CMT using a CMT Kit (Leucocytest®, Synbiotics Corporation, France) to detect subclinical mastitis in four quarters of the udder of cows. The CMT was carried out according to the manufacturer's instructions. The CMT reagent reacts with DNA of epithelial and inflammatory cells found in the milk sample. CMT results were read instantly and were scored for each quarter depending on the thickness and amount of the gel formed. In this study, CMT scores of '0' and trace (±) were held as negative or normal whereas, CMT scores of 1+ (weakly positive), 2+ (distinctly positive) and 3+ (strongly positive) were argued as indicators of subclinical mastitis. In the present study, a subclinical mastitis case was demarked as an animal with at least one of the quarters with a CMT score of ≥1+

Isolation and identification of microbial agents

The milk samples that displayed strong positive reactions (value 3+) in CMT were taken for bacteriological culture. To isolate of bacterial agents from these samples were committed by culturing the milk samples on nutrient agar, MacConkey agar, blood agar and eosin methylene blue (EMB) agar plates. The inoculated plates were incubated at favorable temperature 37 0C under the aerobic condition for 1-2 days. To identify the bacterial agents from the pure culture were performed based on their colony characteristics, Gram staining reaction, hemolysis pattern and biochemical test as described by Cheesbrough (1985).

Statistical analysis

One way ANOVA was used on the treatments of the means of data generated using SPSS 12.0 Windows 2007.

RESULTS

Prevalence of bovine subclinical mastitis

In this study, the whole of 250 cows, 66 (26.4%) was positive to CMT and of 1000 active quarters, 214 (21.4%) were positive to CMT (Table 1). Among 66 CMT positive cases, 19 were strongly positive (score value 3+), 13 were distinctly positive (score value 2+) and 34 were weakly positive (score value 1+).

In the state of, breed-wise prevalence, among the 179 local Cattle (*Bos indicus*), 39 (21.79%) were positive to CMT. Moreover, among 71 crossbreds, 25(35.21%) were positive to CMT. Out of 716 active quarters of *Bos indicus* (Local Cattle) 137 (19.13%)

Table 1: Cattle and quarter-wise prevalence of subclinical mastitis in lactating cows in Bangladesh detected by California Mastitis Test (CMT)

Types	Sample tested	Positive cases	Prevalence rate
Cattle	250	66	26.4%
Quarters	1000	214	21.4%

Table 2**:** Breed and quarter-wise prevalence of subclinical mastitis in lactating cows in Bangladesh detected by California Mastitis Test (CMT)

Types	Sample tested	Positive cases	Prevalence rate
Local Cattle	179	39	21.79%
Crossbreds	71	25	35.21%
Quarters of local zebu	716	137	19.13%
Quarters of Crossbred	284	91	32.04%

Table 3: Prevalence of bovine subclinical mastitis based on various factors

Factors	No. of cows tested (n=250)	Test (n=66)	Prevalence rate
Age			
2-5 years	89	23	25.84%
5-7 years	94	24	25.53%
7-9 years	56	15	26.79%
9-12 years	8	3	37.5 %
>12 years	3	1	33.33%
Breed			
Local Cattle (*Bos indicus*)	179	39	21.79%
Crossbreds	71	25	35.21%
Parity			
1st	79	19	24.05%
2nd	57	13	22.81%
3rd	39	17	43.58%
4th	5	0	0%
5th	45	12	26.67%
6th	18	2	11.11%
7th	7	3	42.86%
Stage of lactation			
Early (15-90 days)	98	26	26.53%
Mid (90-180 days)	71	22	30.99%
Late (>180 days)	81	18	22.22%
Daily milk yield			
0.5-1.5 L	67	22	32.83%
1.5-3.0 L	98	23	23.47%
3-5 L	75	15	20%
5-7 L	8	5	62.5%
7-10 L	2	1	50%
Type of floor			
Factile (Earthen)	104	33	31.73%
Concrete	98	25	25.51%
Slatted	48	8	16.66%

and out of 284 active quarters of crossbreds, 91 (32.04%) were positive to CMT (Table 2).

In case of age-wise prevalence, the prevalence of subclinical mastitis recorded in different age groups was 25.84% (2-5 years), 25.53% (5-7 years), 26.79% (7-9 years), 37.5% (9-12 years) and 33.33% (>12 years). The greatest prevalence based on age groups was 37.5% found in the age group of 9-12 years and the least prevalence was 25.53% found in the age group of 5-7 years (Table 3). The entire prevalence of subclinical mastitis based on lactation stage in cows displayed that all the three lactation stages had subclinical mastitis, notwithstanding, the maximal prevalence of subclinical mastitis was recorded in mid-lactation (30.99%) followed by early lactation (26.53%) and late lactation (22.22%) (Table 3). There was a sharp increase prevalence rate in crossbreds (35.21%) as compared to local (*Bos indicus*) cattle (21.79%) (Table 3). The prevalence of subclinical mastitis related to parity number showed a rising tendency with the

Table 4: Prevalence of microbiall agents isolated from strongly positive subclinical mastitis cases

No. of sample for culture media	Isolation of microbial agent	Number of isolates	Prevalence rate
19	*Staphylococcus spp.*	14	73.68%
	Streptococcus agalactiae	3	10.52%
	Non-aureus Staphylococcus	1	5.26%
	Pseudomonas aeroginosa	1	5.26%
	Actinomyces pyogenes	1	5.26%

rise of parity number. The results of prevalence of subclinical mastitis based on the parity number of cows are presented in (Table 3). Based on parity number of cows, there was dramatic steep prevalence which was 42.86% in 7th parity of cows, while in 2nd and 6th parity, the prevalence was only 22.81% and 11.11%, respectively (Table 3). Based on daily milk yield, the prevalence of subclinical mastitis recorded was 32.83%, 23.47%, 20%, 62.5% and 50%, in lactating cows with the daily milk yield of 0.5-1.5L, 1.5-3.0L, 3-5L, 5- 7L and 7-10L, respectively. The significant prevalence of subclinical mastitis based on daily milk yield was 62.5% showed in the cow milk within a range of 5-7L.

In this study, the prevalence of bovine subclinical mastitis was influenced by the type of floor used for lactating cows. Cows that were sampled having facile (earthen) floor had considerably maximal risks of subclinical mastitis than that of cows reared in the concrete floor system. The amplitude of subclinical mastitis was shot up (31.73%) in lactating cows with a tactile floor as compared to the cows that kept in the house with slatted floors (16.66%) and concrete floors (25.51 %) (Table 3).

Isolation and identification of microbial agent

An entire of 19 milk samples from strongly positive subclinical mastitis cases were examined for the isolation and identification of microbial agents. The results of the isolated microbial agents from strongly positive subclinical mastitis milk samples are illustrated in (Table 4).

DISCUSSION

The overall prevalence of subclinical mastitis was 26.4% in lactating cows at Satkhira district of Bangladesh. These findings almost support the findings of Islam et al. (2011) who reported 29% overall prevalence of subclinical mastitis in lactating cows at Tangail district of Bangladesh. In fact, our findings are much lower than earlier reports of 54.4 to 73.3% (Doguma et al., 2014; Abera et al.2013). This data shows that pure local breeds are more resistant to contracting mastitis than the European. Breed difference in susceptibility to mastitis has also been reported by other studies (Iraguha et al. 2015, Shittu et al. 2012). Likewise, the preponderance of sub-clinical mastitis and its serious economic relevance compared to clinical mastitis was underscored elsewhere (FAO, 2014), Katsande et al.2013, Elbably et al. 2013, Kaliwal et al. 2011). In this research, the prevalence of subclinical mastitis was significantly high in crossbreds (35.21%) as compared to local Cattle (*Bos indicus*) (21.79%).

Kader et al. (2002) reported 46.6% prevalence of subclinical mastitis in crossbred lactating cows of Bangladesh, which is lower than the findings of the present study. According to Seegers et al.(2003), the sub-clinical form is 15 to 40 times more prevalent than the clinical form, and usually precedes the clinical form and is of

long duration and high economic relevance. Thus, it is important to emphasize that the sub-clinically affected animals remain a continuing source of infection for herd mates. These differences in prevalence rates of subclinical mastitis might be due to the difference of breeds of animals, management practices and the tests used for screening of the milk samples.

Age of animal might be an important factor to regulate the prevalence of subclinical mastitis in cattle. In the present study, a trend in the increment in the rate of prevalence of subclinical mastitis was observed at the age of the animal increased (Table 3). The increase in subclinical mastitis with age is consistent with other studies (Ghosh et al. 2004; Kader et al.,2003; Radostits et al. 2000).

In this study, the prevalence of subclinical mastitis was influenced by the length of the lactation period and the highest prevalence based on the length of lactation was 30.99% found in mid-lactation (90-180 days). Perhaps this could be linked to the fact that diapedesis of neutrophils into the mammary gland takes longer time in recently calved cows (Radostits et al. 2007) and increased oxidative stress and reduced antioxidant defense mechanisms during early lactation (Sharma et al. 2011). Belayneh et al. 2013 reported the higher prevalence of mastitis in the late stage of lactation while Mureithi and Njuguna (Mureithi et al. 2016) in Kenya found a significantly higher prevalence in the mid-stage. However, Rahman et al. (1997) reported higher prevalence (34.00%) of subclinical mastitis during the 3rd months of lactation.

In this study, the prevalence of subclinical mastitis was influenced by the parity of the animal and the highest prevalence was 42.86% found in 7th parity of cows. This observation supports the reports of Rasool et al. (1985) and Devi et al. (1997) who reported an increasing prevalence of subclinical mastitis with advancing parity. Rising parity number was also one of the predictors noted to associate with the presence of mastitis. Such scenario has also been documented in a number of studies (Mureithi et al. 2016, Abrahmsen et al. 2014, Katsande et al .2013, Belayneh et al. 2013).

Prevalence of subclinical mastitis was also found to be influenced by farm management system such as floor type used for lactating cows in the study area. Radostitis et al., (2007) stated that the use of adequate amounts of good bedding material will reduce incidence. It is obvious that failure to milk mastitic cows last would favor spread of mastitis pathogens between cows by milker's hands resulting in contagious mastitis (FAO,2014). In this study, the prevalence of subclinical mastitis was peak (31.73%) in lactating cows with a floor, whereas the prevalence was comparatively lower (16.66%) in lactating cows with a slatted floor (Table 3). In this research, Staphylococcus spp. was the most predominant isolation where prevalence was 73.68%, which was followed by *Streptococcus agalactiae* (15.79%) and *Non-aureus Staphylococcus* (5.26%), *Pseudomonas aeroginosa* (5.26%) as well as *Actinomyces pyogenes* (5.26%). The cure rate of *S. aureus* infections with antibiotic therapy during lactation is very low and many infected animals become chronic cases and have to be culled (Kulkarni et al, 2013). The finding of this study is almost similar to the findings of Bitew et al. (2010) who reported 72.2% prevalence *of Staphylococcus spp.* in both clinical and subclinical mastitis.

CONCLUSION

The prevalence of subclinical mastitis in cows at Satkhira district of Bangladesh was 26.4%. What is more, the prevalence of subclinical mastitis in crossbred cattle is shot up than that of local zebu cattle. From the results, it also can be apparent that certain risk factors including age, breed, parity, lactation, floor type, and daily milk yield may have been responsible for high prevalence of subclinical mastitis in lactating cows. Lack of implementation of the routine mastitis prevention and control practices by all of the farms observed and the preponderance of the predisposing factors noted are the principal reasons for the observed prevalence of mastitis in the milk shed. *Staphylococcus spp*. was the

maximal frequent bacterial agents associated with subclinical mastitis in cows in the study area. Besides, the animal health service delivery necessity to focus on regular screening of dairy cows for subclinical mastitis and treating of the cases both lactation and dry period, and provision of advice to cull chronically infected animals. Hygienic management might help in the reduction of the prevalence of subclinical mastitis and for early detection of subclinical mastitis. CMT can be conducted on a regular basis as a control measure. By identifying the causal agent, the best antibiotic could be used to counter a specific bacterium species.

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