Article

Investigation of prevalence and risk factors of subclinical mastitis of dairy buffaloes at Bhola district of Bangladesh

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Abstract: The subclinical mastitis is more serious and is responsible for much greater loss to the dairy industry in Bangladesh. This study was performed to determine the prevalence of subclinical mastitis and identify the associated risk factors in randomly selected 200 lactating buffaloes (192 local and 8 crossbred) at some selected regions of Bhola district, Bangladesh during the period from March 2018 to February 2019. California Mastitis Test (CMT) was performed on each quarter milk sample of lactating buffaloes at field condition for the determination of subclinical mastitis in dairy buffaloes. Overall prevalence of subclinical mastitis was 10.50% (21/200). The prevalence of subclinical mastitis (SCM) was comparatively higher in crossbred (12.5%) as compared to local breed (10.42%). The highest prevalence of SCM in dairy buffaloes was also found in late lactation period (12.82%), age group 7 to 18 years (13.46%), rainy season (11.1%) and 2nd parity group (10.74%). Moreover, the buffaloes graze in free range areas; large and medium sized farms; poor health conditions and other diseases of post parturient period increasing the susceptibility of SCM. For early diagnosis of subclinical mastitis, CMT can be performed regularly as a control measures and emphasis should be provided on farm management practices; particularly on milking hygiene and udder sanitation.

Keywords: prevalence; subclinical mastitis; risk factors; dairy buffaloes

1. Introduction

Bangladesh is a densely populated agro based developing country where livestock plays a crucial role in the national economy. About 1.54% of the total Gross Domestic Product (GDP) is contributed by livestock (BBS, 2018). The majority of the dairy cattle belong to small holder dairy producers. Annual milk production in Bangladesh is 9.4 million metric tons and about 95% milk comes from cattle (DLS, 2017-18). However, Bangladesh has an acute shortage of milk. In present, the produced milk can fulfill only 13.6% of the total requirement in Bangladesh. Dairying in Bangladesh is growing faster, but it also faces lots of problem including high input cost and low output prices. The diseases along with non-availability of feed resources and nutrition are the most important constraints to milk production. Among the diseases of dairy cows, mastitis is one of the most important infectious diseases. The primary cause of mastitis in cattle, buffaloes, goats and sheep are well-recognized groups of microorganisms. Among the microbes *Streptococcus* sp*., Staphylococcus* sp*, Pasteurella* sp*.,* and coliforms*, Escherichia coli, Enterobacter* sp*., and Klebsiella* sp. are the common mastitis causing pathogen. Yeast and fungus have also been found frequently infecting the udder (Andrews *et al.*, 1985). It has many negative economic impacts on dairy farms in terms of abnormal milk, reduced production, deterioration of milk quality and treatment cost (Seegers *et al*., 2003). The disease causes huge economic loss to the dairy industry and the annual economic losses due to mastitis have been estimated to be Tk.122.6 (US$ 2.11) Million

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(Kader *et al.*, 2002). Early recognition and prompt treatment are important for limiting tissue damage and production losses. However, a large proportion of mastitis, glands are not readily detectable by manual palpation or by visual examination of the milk using a strip cup. In the present state of knowledge, it seems practicable and reasonable to define mastitis as a disease characterized by the presence of significantly increased leucocytes content in the milk from affected gland (Blood *et al*., 1983). The disease is common in high yielding dairy cows and buffaloes. Infection rate is more in successive lactation than the first lactation. Exotic and cross bred cows and buffaloes are more prone to mastitis. It is well known fact that the sub clinical mastitis (SCM) is more serious and is responsible for much greater loss to the dairy industry in Bangladesh. The prevalence of clinical mastitis in dairy cows of Baghabarighat, Sirajgong has been reported 16% (Kabir *et al*., 2017). It has been reported that prevalence of sub clinical mastitis in milch cows 16.52% with White side test (WST) and 15.77% with California mastitis test (CMT) from Baghabarighat, Sirajgong district by Prodhan *et al*., (1996) and 18.5% with WST from the Mymensingh district by Rahman *et al*. (1997). Animal breed, farm type, parity of cows, physiological status of animal and milk yield per animal are also found to be important risk factors associated with the occurrence of subclinical mastitis in dairy animals. Khan and Muhammad (2005) reported that overall prevalence of clinical and subclinical mastitis were 5.5 % and 36 % (crossbred cows), 4.0 % and 27 % (buffaloes), respectively. Bilal *et al*. (1999) also reported 16.72 and 21.08% clinical mastitis among cattle and buffaloes, respectively. Ali *et al.* (2011) reported that subclinical mastitis is a great problem in dairy buffaloes in Pakistan which hamper the total milk production of the country. They also found that 44% of dairy buffaloes are affected with subclinical mastitis in some selected regions of Pakistan. Tripura *et al.* (2014) revealed the prevalence of subclinical mastitis in lactating cows was 51.8%, of which 51.4% at Mymensingh sadar and 52.2% at Lakshmipur sadar upazilas of Bangladesh. It has been reported that highest prevalence of mastitis in buffaloes as 58% in backyard management system followed by small holdings in periurban area (42%) and the lowest at organized farms with reasonable good management conditions (32%) in Pakistan (Ali *et al*. 2011). Higher incidence of the disease was observed during winter (18.8 %) followed by summer (16.0 %) by Malik and Haq (2009). They also reported that prevalence rate of bovine mastitis in Kashmir valley was 34.0 and 15.4 % in organized and un-organized farming conditions, respectively. Islam *et al.,* (2011) found higher prevalence of SCM in milch crossbred cows (36.36%) in comparison to local bred cows (24.61%) maintained under extensive management system in rural area of Tangail sadar upazila. The prevalence of sub-clinical mastitis in cow was significantly higher in 3rd (80%) parity compared to 1st (38.09%) and 2rd (45.83%) parity (Sarker and Biswas, 2017). Hygienic milking in cows and buffaloes related to the subclinical mastitis of the individuals. Deng and Asebe (2015) in Ethiopia showed that washing udder was not practiced by 95.17% of the households, which increases the susceptibility of infection. The literature so far available revealed that no study has been carried out on the prevalence of sub clinical mastitis in dairy buffaloes in Bangladesh. So, present study was performed to know the overall prevalence and associated risk factors of subclinical mastitis in dairy buffaloes at selected study area.

2. Materials and Methods

2.1. Study area and duration
The study was carried out in different selected areas of Bhola district, Bangladesh. The study was conducted during March 2018 to February 2019.

2.2. Study design and data collection
Data were collected from the farmer through questionnaire method. Personal interview of the farmers as well as history of farm will be taken.

2.3. Sample Size and milk sample collection
A total of 200 buffaloes were examined throughout the study time to determine the prevalence of sub clinical mastitis. The sample tube (15 ml) were washed with tap water, air dried, plugged and sterilized at 160◦C in the hot air oven for one hour. The tubes were then labeled indicating the dairy farm, name or number of animal and collection date. Milk samples were collected from buffaloes after washing with clean water along with antiseptic wash of teats with 70% ethanol and dried of by tissue paper. Two streams of milk were discarded and then 8 ml of milk were taken aseptically into labeled sterilized test tubes with rubber cap at the time of morning milking.

2.4. Detection of subclinical mastitis
Immediately after collection of milk, samples were subjected to California mastitis test (CMT). The test procedure are described as below-

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2.4.1. California Mastitis Test (CMT)

CMT kit was supplied by ACI Agro vet division, Bangladesh. It is composed of Alkyl Aryl sulfonate (3%), sodium hydroxide (1.5%) and bromocresol purple (1:10,000) as an indicator. For conducting the CMT, a shallow paddle having four cups was used and rinsed after each use. About 5 ml milk was drawn from bottle into the cup (Figure 1) and an estimated equal volume of CMT reagent was squirted from a polyethylene wash bottle. Mixing was accomplished by gentle circular motion of the paddle in a horizontal plane (Figure 2). The reaction developed almost immediately with milk containing a high concentration of somatic cells (Figure 3). The peak of reaction was obtained within 10 seconds and scored.

Interpretation of CMT test:

Severity of infection	Score
Severe	+++
Moderate	++
Mild	+
Absent	-

2.5. Statistical analysis

Prevalence was calculated as the number of positive cases of subclinical mastitis in total collected samples. Data also analyzed by Chi-square test to observe the significant influence of age, parity, stage of lactation, health status, hygienic management, season etc. on subclinical mastitis of buffaloes.

Figure 1. Milk sample from four different teats.

Figure 2. Mixing of reagent and sample.

Figure 3. Gel formation of sample in positive cases.

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3. Results

3.1. Prevalence of subclinical mastitis in buffaloes according to host factor

The overall prevalence of subclinical mastitis as 21 (10.5%) from 200 dairy buffaloes. A total of 20(10.42%) local breed dairy buffalo out of 192 were found to be positive where 1(12.5%) cross breed out of 8 tested animals gave positive result shown in Table 1. Among the 21 positive cases from 200 dairy buffaloes, SCM was recorded as 7(7.29%) and 14(13.46%) in the age group of 3 to 6 years and 7 to 1 8 years, respectively shown in Table 1.The parity wise prevalence of SCM was recorded as 10.13% and 10.74 % during the parity numbers 1 and 2, respectively. The present study also showed that the prevalence of subclinical mastitis in dairy buffaloes as 5.88 %, 10.42 % and 12.82 % in early, mid and late lactation, respectively.

3.2. Prevalence of subclinical mastitis in buffaloes according to management factor

The present study showed higher prevalence of subclinical mastitis in dairy buffaloes as 10.99% which were reared in free range grazing area as compared with stall feeding area as 5.56%. The present study also showed that comparatively higher prevalence of subclinical mastitis in dairy buffaloes as 11.51% which are affected with post parturient diseases as compared with none affected as 8.20%, respectively. The present study revealed the prevalence of subclinical mastitis in dairy buffaloes as 11.0 2%, 10.29% and 0 % in poor, medium and good health conditions, respectively. It has also been found that highest prevalence of subclinical mastitis in medium medium sized farms of dairy buffaloes as 11.76 % followed by large (9.71%) and small (8.33%) sized farm shown in Table 2.

3.3. Season wise prevalence of subclinical mastitis in buffaloes

Among the 21 positive cases from 200 dairy buffaloes, 7(10.94%) was recorded in summer, 10(10%) in winter and 4(11.11%) in rainy season, respectively shown in Table 3.

Table 1. Prevalence of subclinical mastitis in buffaloes according to host factor.

Variable	Category	No. of observation	Subclinical mastitis (+) ve N (%)	Chi square value	*P* value
Breed	Local	192	20(10.42%)	0.035	0.851 NS
	Cross	8	1(12.5%)		
Age	Age 3-6 years	96	7(7.29%)	0.022	0.155 NS
	7-18 years	104	14(13.46%)		
Parity	Parity 1 (1-3)	79	8 (10.13%)	0.019	0.889 NS
	Parity 2 (4-next)	121	13(10.74%)		
Lactation period	Early (15-90 days)	17	1(5.88%)	0.610	0.737 NS
	Mid (90-180 days)	144	15(10.42%)		
	Late (>180 days)	39	5(12.82%)		
Total		200	21(10.5 %)		

NS: Non significant at 5% level of probability

Table 2. Prevalence of subclinical mastitis in buffaloes according to management factor.

Variable	Category	No. of observation	Subclinical mastitis (+) ve N (%)	Chi square value	*P* value
Feeding management	Free range area	182	20 (10.99%)	1.116	0.572 NS
	Stall feeding area	18	1(5.56%)		
History of post parturient diseases	Present	139	16(11.51%)	0.495	0.481 NS
	Absent	61	5(8.20%)		
Health status	Poor	127	14(11.02%)	0.627	0.731 NS
	Medium	68	7(10.29%)		
	Good	5	0(0%)		
Farm size	Large (>50)	103	10(9.71%)	0.627	0.731 NS
	Medium (11-50)	85	10(11.76%)		
	Small (1-10)	12	1(8.33%)		

NS: Non significant at 5% level of probability

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Table 3. Prevalence of subclinical mastitis according to the season.

Level	No. of observation	Mastitis (+) veN (%)	Chi square value	*P* value
Summer	64	7(10.94%)		0.973
Winter	100	10(10%)	0.045	**NS**
Rainy	36	4(11.11%)		

NS: Non significant at 5% level of significant

4. Discussion

4.1. Prevalence of subclinical mastitis in dairy buffaloes according to host factor

Among 200 dairy buffaloes, 21 buffalos were found positive with subclinical mastitis in study area. The overall prevalence of subclinical mastitis was recorded as 10.5 % (Table 1). Ali *et al*. (2011) reported overall prevalence 44% in dairy buffaloes at Punjab, Pakistan, which is higher than the reported data. Kader *et al*. (2002) also reported higher prevalence of SCM in dairy cows 46.6%. Recently, Meher *et al.,* (2018) reported overall prevalence of SCM in dairy cows as 28.50%. The variation of prevalence rates of subclinical mastitis in dairy cows might be due to different breeds of animals, management practices, geographical location and the tests used for screening of the milk samples.

In performed study, among 192 local breed 20(10.42%) buffaloes were found to be positive with SCM tests and among 8 Murrah breed 1(12.5%) animals gave positive result shown in Table 1.The present study indicates that prevalence of SCM was comparatively higher in cross bred than local individuals. Islam *et al*. (2011) also found higher prevalence in cross bred than local bred cows in their study, which support the present data. It has been reported that high yielding cows are more prone to udder infection than low producing one (Hossain, 2004 and Radostits *et al*., 2000). Srinivashan *et al*. (2013) also found the same results in their study.

In present study, among the 21 positive cases from 200 dairy buffaloes SCM was recorded as 7(7.29%) and 14(13.46%) in the age group of 3 to 6 years and 7 to 18 years, respectively (Table 1). Islam *et al*. (2011) reported comparatively higher prevalence of subclinical mastitis in dairy cows as 27.94% in the age group 5 to 7 years than age group 8 to 10 years as 18.19% which is disagree with the present data. The dairy cows of 8-10 years age group are yielding low milk production and less susceptible to SCM as compare to other age group. But, Rahman *et al*. (2010) support the present data. The increase in subclinical mastitis with age is consistent with other studies (Kader *et al*. 2003, Ghosh *et al*. 2004 and Radostits *et al*. 2000).

According to Table 1, the prevalence of SCM in dairy buffaloes was recorded as 10.13% and 10.74 % during the parity numbers 1 and 2, respectively. Srinivashan *et al*. (2013) showed the prevalence of SCM in cows was recorded in 17.94%, 20.58%, 23.07% and 32.50% during the parity numbers 1, 2, 3 and 4 respectively which is quite similar to the current study. It has been reported that the prevalence of SCM in cows were recorded as 18.75%, 22.22%, 29.55% and 26.66% during the parity numbers 1, 2, 3 and 4, respectively (Rahman *et al*. 1997). Gebriwahid *et al*. (2012) reported 18.33%, 34.78%, 32.13% and 14.55% prevalence of SCM in 1st, 2nd, 3rd and 4th parity, respectively in dairy cows which is dissimilar with present study. The high parity cows have less defense status as their teat sphincter get loosen and cellular defense lowered; resulting the organism to get easy entrance through the teat canal on the contrary younger cows are less susceptible to mastitis because of their more effective defense mechanism. Polymorphoneuclear leukocytes functions well in primiparous cows than multiparous ones (Roy *et al*., 1998). This result also is in agreement with the observation made by Kapur and Singh,(1975) who reported comparatively higher incidence rate of mastitis in 2nd parity than 1st one. The finding of present study also support with the findings of Rasool *et al.,* (1985). They also reported that cows had an increase trend of mastitis, as the parity increase.

The present study showed that the prevalence of subclinical mastitis in dairy buffaloes as 5.88%, 10.42% and 12.82 % in early, mid and late lactation, respectively (Table 1). Deng and Asebe (2015) reported 56.52%, 64.71% and 57.45% in early, mid and late lactation, respectively which is slightly similar to the present study. Srinivashan *et al*. (2013) also reported similar observation as reported by Gebriwahid *et al*. (2012).

4.2. Prevalence of subclinical mastitis in dairy buffaloes according to management factor

The present study showed higher prevalence of subclinical mastitis in dairy buffaloes as 10.99% which were reared in free range grazing area (non organized farm) as compared with stall feeding area (organized farm) as 5.56%, respectively shown in Table 2. The present study inclined with the study of Ali *et al*. (2011) where they showed that low prevalence of SCM of dairy buffaloes in organized farm with reasonable good managemental conditions (32%) and high prevalence in non organized peri urban area as 42%.

The present study showed higher prevalence of subclinical mastitis in dairy buffaloes as 11.51% which were affected of post parturient diseases as compared with none affected individuals as 8.20%, respectively shown in Table 2. The result is supported with Rahman *et al.,* (2010) where they also showed that other post parturient diseases of cows increase the susceptibility of subclinical mastitis in dairy cows. The lower immunity level of periparturient cows makes the cow more prone to infection in the udder by Rainard and Riollet, (2006). Calcium ions are necessary for muscle constriction. For this reason, low level of calcium decreases the rigidity of the teat sphincter that might be allows the organism to pass into the udder (Paape and Guidry, 1993). In addition, the cows which were infected with metritis causing release of uterine discharge and retained placenta increase the risk of udder and teats being contaminated (Peeler *et al.,* 1994).

The present study showed that the prevalence of subclinical mastitis in dairy buffaloes as 11.02 %, 10.29% and 0 % in poor, medium and good respectively (Table 2).Usually good health condition of animals is naturally more immunocompetent than poor health individuals. It has been reported that high yielding dairy cows have high non specific udder resistance than low milk yielding cows.

The present study showed higher prevalence of subclinical mastitis in medium sized farms of dairy buffaloes (11.76%) than large (9.71%) and small sized farm (8.33%) shown in Table 2. Ali *et al.* (2011) reported highest prevalence of SCM in individual holding buffalo farms (52%) followed by small holdings (48%) and organized farms (32%) of dairy buffaloes at Punjab, Pakistan, which is dissimilar to present study due to the different regions, therapeutic management of diseased buffaloes, hygienic management of farm and presence of microorganisms in environment. Nooruddin *et al.,* (1997) and Rahman *et al.,* (1997) also reported that percentage of mastitis is higher in large and medium sized farm and support the present study.

4.3. Season wise prevalence of subclinical mastitis in dairy buffaloes

Among the 21 positive cases from 200 dairy buffaloes, 7(10.94%) was recorded in summer, 10(10.0%) in winter and 4(11.11%) in rainy season, respectively shown in Table 3. The occurrence of mastitis varies from season to season, because growth and multiplication of organisms depends on specific temperature and humidity. Incorrect ventilation, with high temperature and relative humidity, encourages the multiplication of various bacteria. Exposure of animals to high temperature can increase the stress of the animal and alter immune functions by Sudhan and Sharma (2010). The dairy cows were more prone to SCM in the monsoon season compared with summer or winter by Joshi and Gokale (2006) which is similar to present study. Dego and Tareke (2003) also showed that prevalence rate is comparatively higher in the rainy season than dry season. Most of the owner of dairy buffaloes in study area avoid dry season as parturition time due to deficiency of grazing land and stress factor which might be involved with reduce the prevalence rate of mastitis in dry season.

5. Conclusions

Mastitis is a common problem of dairy industries. The overall prevalence of subclinical mastitis in dairy buffaloes at some selected regions of Bhola district in Bangladesh was 10.50%. The prevalence of subclinical mastitis (SCM) was comparatively higher in crossbred as compared to local breed. The highest prevalence of SCM in dairy buffaloes was also found in late lactation period, age group 7 to 18 years, rainy season and 2nd parity group of dairy buffaloes. Moreover, the buffaloes graze in free range areas; large and medium sized farms; poor health conditions and other diseases of post parturient period increasing the susceptibility of SCM. Udder hygiene and proper milking management methods, proper installation, function and maintenance of milking equipment, dry cow management and therapy, appropriate therapy of mastitis during lactation, culling chronically infected cows, good record keeping, periodic review of the udder health management will help to take the challenges of mastitis control program. A well-documented continued research and educational effort is required to increase awareness to the farmers or relevant personnel of dairy enterprise. Further study should be performed to determine the specific causal agents, antimicrobials and genetic analysis of the agents to curb the mastitis in mentioned study area.

Acknowledgements

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Conflict of interest

None to declare.

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