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STUDY ON PREVALENCE AND RISK FACTORS OF SUBCLINICAL MASTITIS IN LACTATING DAIRY COWS IN RAJSHAHI AND RANGPUR DIVISION OF BANGLADESH

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A cross sectional epidemiological study was run on subclinical mastitis to determine its prevalence and to identify associated risk factors in total of 261 dairy cows of different breeds and ages distributed in different villages in Rajshahi and Rangpur division of Bangladesh during January 2008 to December 2010 through field screening surveys by using of the California mastitis test (CMT) for each quarter milk sample. The dairy cows were differed from the breed point of view as 147 local Zebu breed and 114 Holstein x local Zebu cross (LxHF) breed. All dairy cows were apparently healthy with clinically sound udder secreting apparently normal milk. Based on CMT results, prevalence of subclinical mastitis was significantly ($p<0.01$) higher in crossbreeds (53.5%) as compared to indigenous Zebu cattle (36.1%) at cow level; and at quarter level 31.6% and 19.2% in crossbreds and local Zebu, respectively. In the present study, some potential risk factors such as age of cows, parity, stage of lactation, body condition in addition to some environmental factors and farm management factors were studied to find out their effects on the frequency of subclinical mastitis of lactating cattle. Among the host related potential risk factors breed, age, parity and body condition were found significantly ($p<0.01$) associated with the high prevalence of subclinical mastitis. Some environmental factors including type of housing, different husbandry systems, periodical disinfection of floor and hand washing before milking also influenced the prevalence of subclinical mastitis in lactating dairy cows. Some recommendations as intervention strategies were forwarded for improved control of mastitis in the region.

Key word: Epidemiology, Subclinical mastitis, California mastitis test (CMT), Intramammary infection, Risk factors.

Although several problems have hindering the livestock development in Bangladesh, but diseases 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 milk is of great financial interest. Milk quality is mainly affected by bacterial contamination of the mammary gland, which causes mastitis (Boscos *et al.*, 1996). Mastitis is one of the major problems of the dairy industry in worldwide including Bangladesh. It is the inflammation of mammary gland affects lactating animals including cattle, goats, sheep, buffaloes and camels and is almost always associated with bacterial infection characterized by swelling, heat, redness, hardness and pain with abnormalities in milk (Fox, 2009). It is the most prevalent production disease in dairy herds and under untreated conditions, it constitutes a serious problem in dairy herds with considerable economic consequences, mainly due to fall in milk production, decreased milk quality for dairy purposes and poor milk hygiene (Seegers *et al.*,2003). Mastitis mainly occurs in two forms i.e. subclinical and clinical (Kader *et al.*,2003). Subclinical mastitis has been found to be 3 to 40 times more common than clinical mastitis (Schultz *et al.,* 1978; Roy et al.,

2009; Mekibib *et al.,* 2010). Subclinical mastitis is considered more severe than clinical mastitis, as early detection is impossible without regular monitoring. Cows with subclinical mastitis harbor a constant reservoir of pathogens that could lead to severe udder infection and spreading to other cows (Philpot and Nickerson, 1999). 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; Bradley, 2002).

Several risk factors such as host related factors(age, parity stage of lactation, body condition), management practices (udder hygiene, husbandry system, floor disinfection and hand washing) and environmental factors (season and housing) have been reported to be important in the prevalence and epidemiology of subclinical mastitis (Bartlett *et al.,* 1992; Chassagne*et al.,* 1998;Almaw*et al.,* 2008).To date however, information is not available on the occurrence of subclinical mastitis nor is there information on the prevalent risk factors for mastitis on dairy farms in the country. The present study was therefore conducted to determine the prevalence of subclinical mastitis and it's associated of risk factors on farms and to assess their relationship to the occurrence of subclinical mastitis in lactating dairy cows.

MATERIALS AND METHODS

The study was conducted from June 2008 to December 2010. Field survey for the prevalence of subclinical mastitis was carried out at all the districts of both Rajshahi and Rangpur division of Bangladesh. The entire laboratory investigations were performed at the Molecular Biology Laboratory of Institute of Biological sciences, University of Rajshahi, Bangladesh. The study was undertaken on dairy lactating cows belonging to government, private and house hold dairy and goat farms in all districts of Rajshahi and Rangpur division of Bangladesh. A total of 261 dairy lactating cows in this observational study were investigated. Among dairy cows there were 147 local zebu cows and 114 high yielding crossbred (LxHF) cows. All dairy cows were apparently healthy with clinically sound udder secreting apparently. All animals were subjected to clinical and physical examinations with special interest towards the udder and teats. Simple random sampling method was considered to select the individual dairy cow. A questionnaire with the primary objective of identifying risk factors for subclinical mastitis was administered at every farm visited. The questionnaire was pre-tested prior to their final use. Some of the factors investigated included farm management and animal husbandry practices including host and environmental factors influencing the incidence of mastitis.

The udders and especially teats were cleaned and dried before sample collection. Each teat end was scrubbed with cotton moistened with 70% ethyl alcohol. A squirt of milk, about 2 ml from each quarter was placed in each of four shallow cups in CMT paddle. Then an equal amount of the CMT reagent was added to each cup. A gentle circular motion was applied to the mixtures in a horizontal plane for 15 seconds. A cow or a quarter was considered to have subclinical mastitis if CMT score is 1, 2 or 3 and the California Mastitis Test (CMT) was carried out according to the method described by Quinn *et al.* (1999).

Statistical analysis

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.

RESULTS

Overall prevalence of subclinical mastitis in cattle

In case of cattle, among 1044 quarters milk of 261 cows were examined for the diagnosis of subclinical mastitis and in cow level subclinical mastitis was found to be 43.7% and in quarter level it was 24.8%.

Prevalence of subclinical mastitis in different breeds of cattle

Both at cow and quarter level, prevalence were high in crossbreds as compared to local breeds. ($p<0.005$) (Table 1).

Prevalence of subclinical mastitis of cattle in Rajshahi and Rangpur division

Subclinical mastitis prevalence did not vary significantly ($P> 0.05$) between the study sites (Table 2).

Risk factors of subclinical mastitis in cattle

In the present study different potential risk factors such as age of cows, parity, stage of lactation, body condition in addition to the environmental factors and farm management factors were studied. These factors were considered in chi-square statistical analysis test to find out their effects on the frequency of subclinical mastitis of lactating cows.

Different host related risk factors affecting subclinical mastitis infection rate

Out of the four potential host related risk factors considered (Table 3) age, parity and body condition were found to be significantly ($p< 0.05$) influenced the rate of prevalence of subclinical mastitis in cattle. The study indicated that subclinical mastitis was significantly influenced by the age, parity and the body condition of cows; whereas, subclinical mastitis was not significantly ($p> 0.05$) influenced by the different stages of lactation of cows.

Environmental factors affecting subclinical mastitis infection in cattle

In this study, it was found that subclinical mastitis was significantly ($p=0.0001$) influenced by the housing of cows; in contrast,subclinical mastitis was not significantly ($p>0.05$) influenced by the seasons of environment (Table 4).

Farm management factors affecting subclinical mastitis infection in cattle

In the present study, subclinical mastitis was not significantly ($p>0.05$) influenced by udder hygiene of cows; whereas, it was significantly ($p=0.0001$) influenced by the different practiced husbandry systems of cow and by the cows reared on floors disinfected periodically or not. The result also showed that subclinical mastitis was significantly ($p<0.05$) influenced by the management practice like hand washing before milking of cows(Table 5).

DISCUSSION

In this study as well as in other similar studies, the majority of the cases of mastitis were subclinical compared to clinical mastitis in both breeds (Kassa *et al.*,1999; Hussein, 1999; Workineh *et al.*,2002; Kerro and Tareke, 2003).

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

Host	Cows			Quarters			Chi-square value	Sig. level
	Total	SCM infected	Infected (%)	Total	No. infected	Infected (%)		
Local Zebu	147	53	36.1	588	113	19.2	7.967	**
Cross (LXHF)	114	61	53.5	456	144	31.6		
Total	261	114	43.7	1044	257	24.6		

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

Table 2: Prevalence of subclinical mastitis in cattle of both Rajshahi and Rangpur division

Study area	Total cows	Subclinical mastitis infected	Infected (%)	Chi-square value	Sig. level
Rajshahi Division	120	56	46.7		
Rangpur Division	141	58	41.1		
Total	261	114	43.7	.806	NS

NS = Not significant

Table 3: Prevalence of subclinical mastitis of cattle in relation to host related factors

Risk factors	Cows			Quarters			Chi-square value	Sig. level
	Total	SCM infected	%	Total (1044)	Infected no.	%		
Age (Years)								
Young adult (>3-6)	108	26	24.1	432	63	14.6		
Adult (>7-10)	66	27	41	264	76	28.8	43.000	**
Old (>10)	87	61	70.1	348	137	39.4		
Parity								
Few(≤3)	163	36	22.1	652	92	14.1		
Moderate(4-7)	83	63	75.9	332	133	40.1	93.850	**
Many(≥7)	15	15	100	60	23	38.3		
Stage of Lactation								
Early(1-4)	97	37	38.1	448	43	9.6		
Mid(5-8)	74	31	41.9	248	67	27	3.321	NS
Late(≥9)	90	46	51.1	348	149	42.8		
Body condition								
Good	91	17	18.7	364	36	9.9		
Medium	62	27	43.5	248	69	27.8	44.984	**
Poor	108	70	64.8	432	154	35.6		

**significant at 1% level (p<0.01)
NS=Not significant

Table 4: Prevalence of subclinical mastitis of cattle in relation to environmental factors

Risk Factors	Cows			Quarters			Chi-square value	Sig. level
	Total (261)	Infected no.	Infected %	Total (1044)	Infected no.	Infected %		
Housing								
Earth floor	190	100	52.6	760	246	32.4	24.271	**
Concrete floor	71	14	19.7	284	37	13		
Season								
Dry season	130	54	41.5	520	126	24.2	.482	NS
Rainy season	131	60	45.8	524	133	25.4		

**significant at 1% level (p<0.01)
NS=Not significant

The overall prevalence of subclinical mastitis at cow level based on CMT in the present study was 43.7 %. Similar prevalence was reported by other scientists such as Mdegela *et al.* (2005) 50%, Mekibib *et al.* (2010) 48.6%; Rahman *et al.*,(2010) 51.3%, and Kinabo and Assey (1983) 40%. The prevalence rates we found in this study are considerably higher compared to the previous reported prevalence rate reported by many other investigators like Hawari and Al-Dabbas (2008), Workineh *et al.* (2002), Almaw*et al.* (2008), Bitew *et al.*, (2010) and Abdel-Rady and Sayed (2009), who reported 31.4, 38.2 , 34.4, 25.2 and 19.14%, respectively.

In some studies a much higher prevalence rate of subclinical mastitis was found as 90.3% (Kivaria *et al.*, 2004), 76%

Table 5: Prevalence of subclinical mastitis of cattle in relation to management factors

Risk factors	Cows			Quarters			Chi-square value	Sig. level
	Total (261)	Infected no.	Infected %	Total (1044)	Infected no.	Infected %		
Udder hygiene								
Washing	83	30	36.1	332	83	25	2.836	NS
Not washing	178	84	47.2	712	176	24.7		
Husbandry system								
Intensive	76	14	18.4	304	32	10.5	37.609	**
Semi-intensive	97	43	44.3	388	107	27.6		
Extensive	88	57	64.8	352	120	34.1		
Floors disinfected periodically	67	13	19.4	268	34	12.7	23.098	**
Yes	194	101	52.1	776	225	29		
No								
Hand washing								
Yes	98	31	31.6	392	69	17.6	9.411	**
N	163	83	50.9	652	190	29.1		

**significant at 1% level ($p<0.01$)
NS=Not significant

(Karimuribo *et al.*, 2008) and 70% (Kapaga *et al*., 1995).

These differences could most probably be attributed to the differences in the distribution of mastitis risk factors may such as experience in dairying, the potential effects of levels of milking hygiene and cleanliness, and the application of sanitary measures such as post-milking teat dipping. As mastitis is a complex disease and the difference in results could be due to the difference in management system between the farms.

Mastitis has a multifactorial nature that predominates with a clear interaction between host, agent and environment (Thrusfield, 2005). For this reason, the studied factors here were determined as hypothesized risk factors affecting mastitis. With studying the breed factor, it is found that the prevalence of subclinical mastitis is significantly ($\chi^2= 7.967$, $P <0.05$) lower in the local Zebu breed of cows (36.1%) than the cross breed (L X HF) cows (53.5%). The present result was in accordance with many other researchers like Dego and Tareke, (2003) in southern Ethiopia, found that indigenous Zebu more resistant than Holstein-Friesian, Mdegela*et al.* (2005) in Tanzania found that traditional animals more resistant than dairy animals. Ghazi and Niar (2006) in Algeria found that local breed more resistant compared to the imported ones. This is primarily due to the genetic resistance (Payne and Wilson, 1999), the inherent low milk productivity and also to the bad adaptation of these cows to local environment and climate. However, more studies are needed to shed more light on this differential udder infection rates between local and cross breeds.

The significantly increased prevalence of mastitis with age and parity reported in the present study is in agreement with the report of Biffa *et al.* (2005).This also agrees with Rahman*et al*. (2009) who had reported prevalence of mastitis as 12.3% and 65% during the first and last parity respectively. The age-multiparous-high prevalence is explored to be due to all increase in teat patency and degree and frequency of previous exposure in multiparous old cows (Harmon, 1994; Radostits *et al*., 1994).According to Erskine (2001),

primiparous cows have more effective defense mechanism than multiparous cows. Although there was no significant association between the prevalence of subclinical mastitis and stage of lactation (χ^2 = 3.321, P >0.05), these results are inconsistent with previous reports (Biffa *et al.,* 2005; Radostits *et al.,* 1994; Kehrli and Shuster, 1994).

In this research, prevalence of subclinical mastitis is significantly associated with body condition of cows studied (χ^2= 44.984, P < 0.001), as many as 64.8% of cows with poor body condition had subclinical mastitis compared with only 18.7% of cows with good body condition. This study is in agreement with the results reported by many other researchers (Sviland and Waage, 2002; Mungube *et al*., 2004). This may be due to the much weaker defense mechanism of poor body condition group of cows. In the present study, there was significant difference detected in the prevalence of subclinical mastitis amongst cows managed intensively, semi-intensively or extensively. The present result is in agreement with the report of Smith and Hogan, (1993) and Schukken*et al.* (1991). These differences may be due to that in semi-intensive and extensive dairy farms, cows were maintained in dirty and muddy common barns with bedding materials that favour the proliferation and transmission of mastitis pathogens.

Of all the management factors investigated in this present study, prevalence of subclinical mastitis is not significantly associated with udder hygiene of cows (χ^2= 2.836, P >0.05). This observation is in agreement with the findings of Hutabarat *et al.,* 1985.

There was also significant association observed between prevalence of subclinical mastitis and hand washing before milking of cows. It was found that cows of hand washing before milking group had a significantly (χ^2= 9.411, P <0.05) low subclinical mastitis infection rate (31.6%) as compared to cows of not hand washing before milking group (64.8%). This observation is in accordance with a previous report of Hutabarat *et al.,*(1985)who found a significant association between hand washing before milking of cows and udder infection status. In this investigation, subclinical mastitis was also significantly (χ^2= 23.098, P < 0.001) more prevalent on farms where periodical disinfection of floor is practiced as compared to those farms with floor not disinfected periodically. This report in the present study is inconsistent with the report of Hutabarat *et al.* (1985), that could partly be explained by the fact that although some farmers disinfect their farm yard periodically but they do not disinfect it properly or the chemical disinfectants usually they use are not of good qualities. In the present study prevalence of subclinical mastitis also significantly (χ^2= 24.271, P < 0.001) higher on farms with earthen floor (52.6%) compared to those farms with concrete floor (19.7%). This observation was similar to the finding of the Mekibib*et al.*(2010). The fact that the majority of the animal houses in our country had earth type of floors which is difficult to clean and the dirty, wet bedding, which is a common finding on earthen floors, tends to harbor a variety of infectious agents, which may contaminate the udder and the teats. Wet bedding also serves as a media in which pathogenic organisms grow vigorously and multiply and therefore may serve as a preservation media for these organisms. In this research, prevalence of subclinical mastitis in both cattle and goats is not significantly (χ^2= .482, P >0.05) associated with season of the year. Cows in rainy season had a subclinical mastitis infection rate 45.8% and in dry season subclinical mastitis infection rate was 41.5%. However, in rainy season had a subclinical mastitis infection rate 57.8% and in dry season subclinical mastitis infection rate was 32.9%. This may be due to lack of proper rainfall and change of climatic condition in Bangladesh now. In contrast mastitis occurrences were highest during the wet season (rainy season) in many countries such as Thailand (Rojstien *et al.,* 2004), India (Joshi and Gokhale, 2006), Brazil (Costa *et al.,* 1998) and Israel (Shpigel *et al.,* 1998).

CONCLUSION

Subclinical mastitis is highly prevalent (43.7%) in cattle of Bangladesh. Local

breeds were found more resistant to subclinical mastitis as compared to high yielding cross breeds in both cattle. More efforts are needed to improve the general udder health in dairy herds, as the overall high prevalence (43.7%) of subclinical mastitis in the present study would threaten the growing dairy industry. The recommended preventive and control measures against mastitis should include application of good sanitary and hygienic measures, providing good housing and culling of aged dairy cows.

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