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Research Article

Comparison between California mastitis test and somatic cell count as diagnostic tools for bovine subclinical mastitis

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Abstract

Mastitis is one of the major diseases affecting lactating animals. The objectives of this study were to determine the prevalence of subclinical mastitis in cows by California mastitis test (CMT) and to compare the CMT results with somatic cell count (SCC) in selected areas of Satkhira district in Bangladesh. A total of 151 cows and 604 quarters of mammary glands derived from 68 small and medium farms were examined by CMT. To compare the CMT results with SCC, somatic cells in the same milk samples of 297 quarters were examined by the Nucleo Counter SCC-100 machine. The CMT results showed that 42.4% of examined cows were positive where one or more quarters had subclinical mastitis. Moreover, 16.2% of tested quarters had subclinical mastitis as diagnosed by CMT. When SCC was done, median values of CMT negative, trace, moderate, distinct and strong CMT score were 84000, 559000, 1203000, 1850000 and 1755000 per ml of milk, respectively. The SCC had a strong positive correlation with the CMT score (r=0.9602). The sensitivity, specificity and accuracy of CMT compared with SCC were 40.7, 96.7 and 69.4%, respectively. Results indicate that both CMT and SCC can be used as tools for the diagnosis of subclinical mastitis in cows.

Keywords: Bovine, CMT, Somatic cell count, Sub-clinical mastitis

INTRODUCTION

Mastitis is the most common and costly production disease in dairy farms worldwide, especially in its subclinical form (Seegers *et al.*, 2003; Halasa *et al.*, 2007). It causes losses due to reduction of milk yield and cost of therapy, unused milk during the withdrawal period and other inputs used to control mastitis (Atasever and Erdem, 2009). It is found that economic losses from clinical mastitis range from €61 to €97 per cow on a farm worldwide; nevertheless, there may be significant variances between farms; as in the Netherlands losses ranged from €17 to €198 per cow per year (Hogeveen *et al.*, 2011). However, the actual losses from mastitis in Bangladesh have not yet been known. Mastitis deteriorates milk quality, as it decreases nutrient contents, alters the flavour and increases microbial loads in milk.

Mastitis control is very important not only to reduce its incidence in dairy animals but also to reduce public health hazards. A systemic or mammary gland infection in the host animal might alter the contents and nutritional values of milk, which are vital for human nutrition. Public health hazards can be caused by the consumption of infected milk and milk contaminated by drug residues that come from the treatment of mastitis. In context to the expanding dairy industry in Bangladesh, it is crucial to develop a quick mastitis diagnosis tool and control programme. The farmer can

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detect clinical mastitis easily, but only the detection of subclinical mastitis requires the measurement of inflammatory components and pathogens in the milk (Nielen *et al.*, 1992). California Mastitis Test (CMT) is one of the effective tests on the cow side for the determination of subclinical mastitis. However, CMT is an indirect method and it gives preliminary information about mastitis. On the other hand, somatic cell count (SCC) is more direct and specific, and it gives a clear reflection on the status of udder health of dairy animals. Skrzypek *et al.* (2004) have stated that high SCC in milk degrades the physicochemical qualities of milk and dairy products, and also affects the shelf life and flavour of milk. Durr *et al.* (2008) state SCC in milk as an effective technique for detecting subclinical mastitis in dairy cows. Moreover, SCC in individual milk and bulk tank is the quick and established method to identify subclinical and clinical mastitis.

By using research data, countries with developed dairy industries consider milk with $\leq$ 200000 somatic cells per ml as normal and milk with $>$ 200000 cells per ml is considered as abnormal milk from mastitis cows (Ruegg and Reinemann, 2002). However, the breed of cows and management practices in those countries may differ from Bangladesh. In Bangladesh, the dairy cows are mostly indigenous and crossbred, and the SCC in their milk is not known which may differ. Therefore, it is essential to determine the SCC in the milk of both normal and mastitis cows in Bangladesh. Estimation of SCC in normal and mastitis milk will help us to develop an effective guideline for a nationwide mastitis control programme. Considering the above-mentioned facts, the study was conducted to determine the prevalence of subclinical mastitis by CMT and to compare the CMT results with SCC in the selected area of Satkhira district in Bangladesh.

MATERIALS AND METHODS

Study areas

The study was carried out at Satkhira Sadar, Debhata and Ashashuni Upazila of Satkhira district, Bangladesh during the period from May to October 2013. The smallholder dairy farmers in these areas are being provided with subsidized productivity veterinary services by the Community-based Dairy Veterinary Foundation (CDVF), Bangladesh Agricultural University, Mymensingh, Bangladesh.

Study population and management of cows

A total of 68 smallholding and medium-size dairy farms were selected randomly in this study. All lactating (n=151) cows from selected farms were tested for subclinical mastitis. The cows were kept in stalls and fed mostly straw, concentrates, and a small number of green fodders. Anthelmintics and vaccinations were given to the cows regularly. The cows were hand milked twice daily keeping their calves at feet; however, during the last part of lactation, many farmers milked their cows once daily.

California Mastitis Test (CMT)

For determination of subclinical mastitis, 604 quarters of 151 lactating cows were examined by CMT. The CMT was performed using 10% Teepol (Leucocytest®, Rhone Meriux, Lyon, France) according to the instruction of the manufacturer. Briefly, a plastic paddle with four receptacles was used for this purpose. After cleaning the teats, 2 ml of foremilk was stripped from 4 teats of each cow separately into the respective cup of the paddle. An equal amount (2 ml) of reagent was added to milk in each cup of the plastic paddle. Then the reagent and milk were mixed in the cups of the plastic paddle by a swirling motion. The result was evaluated immediately by visual examination. Results were recorded as follows:

Negative	when the mixture remained fluid without thickening or gel formation.
Trace	when slight slime formation was observed.
Moderate	when slime formation occurred immediately after mixing the solution and milk and this slime sometimes dissipated.
Distinct	when slime formation occurred immediately after mixing the solution with milk.
Strong	when distinct slime formation occurred immediately after mixing the solution with milk.

Collection of milk samples for somatic cell count (SCC)

For the collection of milk samples, udder and teats were prepared according to the National Mastitis Council guideline (Barkema *et al.*, 1999). The sample was collected before milking in a screw cap 10 ml glass vial

first from the near teats then from the far ones. Udder quarters were washed with tap water and dried. Cotton soaked in 70% ethyl alcohol was used to clean the teat end. Direct streams of about 5 ml milk were collected into the vial after voiding several stripping of milk. Samples were stored in a cool box at 4°C immediately after collection followed by storing within the refrigerator at 4°C for 1 to 5 days. The cooled samples were transported to the laboratory at 4°C by using the cool box.

Somatic cell count (SCC)

The SCC was done for 297 quarter milk samples derived from 81 cows. Somatic cells in milk samples were counted by the Nucleo Counter SCC-100 machine (Chemometec, Denmark). The Nucleo Counter is an integrated fluorescence microscope designed to detect signals from the fluorescent dye, propidium iodide (PI) bound to DNA. Results from the Nucleo Counter represent the total cell concentration. Briefly, 500 µl of milk sample and an equal volume of reagent-C (lysis buffer) were taken into the tube by micropipette and mixed properly. After mixing, the cassettes (disposable and work as calibrated reading cells) were loaded with the cell lysate. After placing the cassette inside the equipment and pressing on the command key, the result appeared on the display and it was printed within 30 seconds. The analysis was completed in 3 simple and fast steps, viz. i) introduction of the sample in the cassette, ii) positioning of the cassette into the instrument and iii) pressure on the "RUN" key. Finally, the digital readings were documented for further analysis. The measurement range was 10000-2000000 cells per ml.

Sensitivity, specificity and accuracy of CMT

Milk samples were considered positive for mastitis when CMT showed positive reaction with reagent and SCC value was $>$ 200000 (threshold value). The following diagnostic test characteristics were determined using the SCC result as a gold standard control.

$$\text{Sensitivity} = \frac{TP}{TP+FN} \times 100$$

$$\text{Specificity} = \frac{TN}{FP+TN} \times 100$$

$$\text{Accuracy} = \frac{TP+TN}{TP+FP+TN+FN} \times 100$$

TP- true positive, FP- false positive, TN – true negative, FN – false negative.

Statistical analysis

Descriptive statistics on SCC was performed using an MS Excel worksheet. Regression-correlation analysis was done by SPSS 11.5 software to determine the relationship between CMT score and SCC. The percentage of sensitivity, specificity and accuracy of CMT result compared to SCC result were calculated using a standard two-by-two contingency table.

RESULTS

A total of 151 dairy cows from 68 farms were examined by CMT for determination of the presence of subclinical mastitis. The results of the CMT of tested cows are presented in Table 1. Among the tested cows, 42.4% (64 out of 151) cows were affected with subclinical mastitis where one or more quarters were affected. When the severity of subclinical mastitis was classified, milk of 9.3% of cows showed trace reaction, milk of 21.2% of cows showed a moderate reaction, milk of 6.6% of cows showed distinct reaction and milk of 5.3% cows showed a strong reaction to CMT.

A total of 604 quarters of 151 dairy cows were examined by CMT for determination of the presence of subclinical mastitis. The results of the CMT of tested quarters are presented in Table 2. Among the tested quarters, milk of 16.2% (98 out of 604 quarters) was affected with subclinical mastitis. When the severity of subclinical mastitis was classified, milk of 3.0% quarters showed trace reaction, milk of 9.4% quarters showed a moderate reaction, milk of 2.5% quarters showed distinct reaction and milk of 1.3% quarters showed a strong reaction to CMT.

The SCC of milk of 297 quarters was determined for comparison of CMT results with SCC. The results on SCC are presented in Table 3. The median SCC values of milk with negative, trace, moderate, distinct and strong CMT reactions were 84000, 559000, 1203000, 1850000 and 1755000 per ml of milk, respectively.

The correlation between CMT score and SCC of quarter milk samples is shown in Figure 1. The SCC of quarter milk samples had significant positive correlation with the CMT score (r = 0.9602). The sensitivity, specificity and accuracy of CMT were determined by comparing the results with that of SCC. The sensitivity, specificity and accuracy of CMT were found 40.7, 96.7 and 69.4%, respectively (Table 4).

Table 1: CMT score of tested milk of cows

CMT score	**The total number of cows examined**	**Cows affected with subclinical mastitis**	
		Number	**%**
Trace		14	9.3
Moderate		32	21.2
Distinct		10	6.6
Strong		8	5.3
Total	151	64	42.4

Table 2: CMT score of tested quarter milk of cows

CMT score	**The total number of cows examined**	**Cows affected with subclinical mastitis**	
		Number	**%**
Trace		18	3.0
Moderate		57	9.4
Distinct		15	2.5
Strong		8	1.3
Total	604	98	16.2

Table 3: Comparison between CMT score and SCC of tested quarter milk

CMT score	**Number of quarters examined**	**Somatic cell count (per ml)**		
		Median	**Minimum**	**Maximum**
Negative	233	84000	10000	2000000
Trace	12	559000	369000	1846000
Moderate	38	1203000	20000	2000000
Distinct	11	1850000	200000	2000000
Strong	3	1755000	1193000	2000000

Table 4: Sensitivity, specificity and accuracy of CMT

		SCC				
		Positive	**Negative**	**Sensitivity (%)**	**Specificity (%)**	**Accuracy (%)**
CMT	Positive	59 (TP)	5 (FP)	40.7	96.7	69.4
	Negative	86 (FN)	147 (TN)			

TP =True positive, FP = False positive, FN = False negative, TN = True negative and number of total CMT positive samples = 64, CMT negative = 233; number of SCC positive sample = 145, negative = 152.

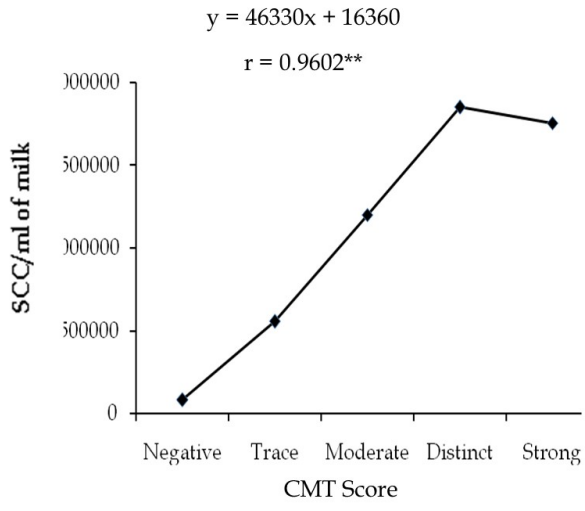

Figure 1: Comparison of CMT with SCC

DISCUSSION

The study evaluated two diagnostic procedures, CMT and SCC, and estimated the prevalence of subclinical mastitis in cows and quarters levels. Using specific parameters of the SCC or CMT score is the quickest and easiest technique to identify intramammary infections (IMIs) in dairy cows. The CMT is considered a practical and easy method for on-farm milk sample testing to identify IMIs (Dingwell *et al.*, 2003). Moreover, it is a rapid and low-cost examination used to indirectly assess the SCC concentration in milk (Midleton *et al.*, 2004).

As per CMT results, overall, 42.4% of examined cows were affected with subclinical mastitis in the present study. Similar to the present study, 44.8% (Rahman *et al.*, 2009) and 42.7% (Sinha *et al.*, 2011) prevalence of subclinical mastitis have been observed in Sirajganj district of Bangladesh. In contrast to this finding, subclinical mastitis was recorded in 48.5% of dairy cows in the United States (Wilson and Gonzalez, 1997), 57.0% in BAU Dairy farm (Kader *et al.*, 2002), and 63.3% in Sarajevo, Bosnia-Herzegovina (Varatanović *et al.*, 2010). Furthermore, earlier research of Chowdhury (2011) in Bangladesh reported a lower rate of subclinical mastitis (15.1%) in cows. However, this variation in the prevalence of mastitis between studies may be due to variation of herd size, agro-climatic conditions of the regions and farm management.

In the present study, according to the CMT score, when subclinical mastitis was classified, the highest proportion (21.2%) of cows were suffering from moderate and the lowest proportion (5.3%) of cows were suffering from strong subclinical mastitis. Similarly, the highest proportion of cows (29.3%) was suffering from moderate and the lowest proportion (2.6%) of cows was suffering from strong subclinical mastitis elsewhere (Varatanović *et al.*, 2010). This indicates that hygienic practices in farms might be compromising resulting in the continuous presence of organisms in the udder although it did not cause the clinical manifestation of mastitis which needs to be improved.

When CMT results for quarter samples were considered, 16.2% of quarters were affected with subclinical mastitis in the present study. Contrasting to this finding, a higher proportion of quarters are found to be affected with subclinical mastitis in crossbred dairy cows in Chittagong district of Bangladesh (27.0%) (Jha *et al.*, 2010), in India (67.8%) (Sharma *et al.*, 2010) and 56% in elsewhere (Ogola *et al.*, 2007). When CMT positive milk samples were classified concerning quarter level subclinical mastitis, it was found that the highest proportion of quarters (9.4%) had a moderate CMT score and the lowest proportion of quarters (1.3%) had a strong CMT score in the present study. Whereas, Bhutto *et al.* (2012) reported 20% of subclinical mastitis affected quarters with a trace CMT score and 5% of quarters affected with a strong CMT score. Kasikci *et al.* (2012) have also found 66.9% of quarters with CMT + positive score and 11.1% of quarters with CMT +++ positive score in dairy cows. The variations among studies may be due to variations in hygienic practices in farms, management of farms and agro-climatic location of farms among studies.

Somatic cells are always present in milk and vary from 50000 to 100000 cells/ml SCC in healthy cow's milk (Kasikci *et al.*, 2012). They increase due to mammary gland infections. If the SCC exceeds 200000 cells/ml, it is considered unhealthy for consumers (Skrzypek *et al.*, 2004). In the present investigation, the median value of SCC in quarter milk samples were 84000, 559000, 1203000, 1850000 and 1755000 per ml in CMT negative, trace, moderate, distinct and strong samples, respectively. Kasikci *et al.* (2012) showed mean SCC values of 249453, 1167058 and 2108139 cells per ml in moderate, distinct and strong CMT reactive milk samples, respectively. Pradiee *et al.* (2012) also reported the median value of 62000 and 123000 cells per ml of milk in CMT negative and positive samples in ewes. Additionally, Başţan *et al.* (2008) reported that SCCs were 100000-200000, 200000-300000, 300000-1000000 and above 1000000 cell/ml, respectively, in the groups of CMT negative, CMT +, CMT ++, and CMT +++ positive milk samples. Further, mean SCCs were 313001, 559007 and 1563618 in CMT +, CMT ++ and CMT +++ positive milk samples, respectively elsewhere (Risvanli and Kalkan, 2002). Bhutto *et al.* (2012) also got $< 150 \times 10^3$, $151-250 \times 10^3$, $251-500 \times 10^3$, $501-750 \times 10^3$ and $751- > 1000 \times 10^3$, respectively for CMT –ve, CMT +, ++, +++ and ++++, respectively by investigating 960 milk samples. The reasons for variations in SCC among studies might be due to variations in methods and machines used for SCC and classification of subclinical mastitis among studies. SCC will likely be increased with a load of organisms in the udder. Accordingly, in the present study, a positive correlation was found between CMT and SCC. A similar positive correlation between CMT and SCC is also observed in dairy cows in Turkey (Kasikci *et al.*, 2012) and India (Sharma *et al.*, 2010).

Early detection and prevention of subclinical mastitis should be a top concern for all dairy owners to assure the acquisition of high-quality milk and minimize financial losses. Although CMT is simple to use in the field, it was not found to be as sensitive as SCC for diagnosing subclinical mastitis in dairy cows in this investigation. This is in agreement with earlier report by Sharma *et al.* (2010) in India. Pradiee *et al.* (2012) also demonstrated that the sensitivity of CMT is lower as a mastitis diagnosis tool in ewes compared to bacterial culture. However, the low cost for examination and easiness of its application by the farmers may make CMT advantageous over SCC for usage in smallholding dairy farms in Bangladesh.

CONCLUSIONS

Both CMT and SCC can be used as tools for the diagnosis of subclinical mastitis in cows. However, more samples should be evaluated for standardization of SCC by an automatic Nucleo Counter machine for accurate diagnosis of subclinical mastitis in cows.

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