

Prevalence of Subclinical Caprine Mastitis in Bangladesh Based on Parallel Interpretation of Three Screening Tests

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Abstract: The present study was conducted to determine the point prevalence of subclinical caprine mastitis based on parallel interpretation of results of three screening test. A total of 462 milk samples from 231 lactating Black Bengal goats comprises of three organized goat farms in Bangladesh were collected and were screened for subclinical mastitis using California Mastitis Test (CMT), White Side Test (WST) and Surf Field Mastitis Test (SFMT) simultaneously. Integrated test results yield the prevalence of caprine subclinical mastitis as 44.59% (95% Confidence Interval of 40.12-49.15%) based on “the OR rule” and 40.48% (95% Confidence Interval of 36.10-45.01%) based on “the AND rule” of parallel interpretation. Animal level and udder half level prevalence of subclinical caprine mastitis were 39.83, 38.96, 38.10% and 35.05, 34.85, 31.60% by CMT, WST and SFMT, respectively noticed when tests were interpreted individually. CMT in combination with one or two other indirect low cost indirect tests can be recommended for screening of caprine mastitis in field condition with “the OR rule” of parallel interpretation.

Keywords: CMT, goats, mastitis, prevalence, SFMT, WST

INTRODUCTION

Mastitis or inflammatory condition of the udder is a common disease affecting dairy goats which is accompanied by physical, chemical, pathological and bacteriological changes in milk and glandular tissue (Samad, 2008). Mastitis in goat is mainly of sub-clinical type (MacDougall *et al.*, 2001), with coagulase negative Staphylococci being the most prevalent isolates (Contreras *et al.*, 2007). In case subclinical mastitis, milk appears normal with no visible abnormalities in udder tissue except an elevated Somatic Cell Count (SCC) in milk which is commonly used as a milk quality standard at the individual animal and herd level (McDougall *et al.*, 2001). Sub-clinical mastitis is important due to the fact that it is 15 to 40 times more prevalent than the clinical form, is of long duration, difficult to detect, adversely affects milk quality and production of dairy animals and constitutes a reservoir of microorganisms that can affect other animals within the herd due to its contagious nature (Schultz *et al.*, 1978).

Early diagnosis of subclinical mastitis is vital because changes in udder tissue take place much earlier than they become apparent. Various approaches, based on physical and chemical changes of milk and cultural isolation of organisms (gold standard), are practiced for diagnosis of sub clinical mastitis in goat (Contreras

et al., 2007). Paape *et al.* (2001) explored the feasibility of diagnosing mastitis in small ruminants by indirect determination of the number of somatic cells in milk. Among the indirect tests, CMT, WST, SFMT are commonly used for indirect somatic cell count as indicator of subclinical mastitis both in cows and does. California Mastitis Test (CMT) is a simple, market available, rapid screening test for sub clinical mastitis, based upon the amount of cellular nuclear protein present in milk sample that react with CMT reagent (Contreras *et al.*, 1995). White Side Test (WST) is an indirect, easily applicable screening test for sub clinical mastitis in which 4% sodium hydroxide solution are used as reagent (Kahir, 2006). Surf Field Mastitis Test (SFMT) is another simple inexpensive indirect test for detection of SCM using 3% solutions of household detergent (Muhammad *et al.*, 1995).

Because of logistic and financial considerations involved, any one of these indirect tests (CMT, WST and SFMT) usually used singly for screening followed by bacteriology for confirmation and for serial interpretation to be made. However, combination of these cost effective tests may increase discriminatory capacity and sensitivity of combined test as well. Therefore, the present study was conducted to determine the mastitis prevalence status in lactating does based on parallel interpretation of three screening tests used combine.

MATERIALS AND METHODS

The present study was conducted on total 462 milk samples collected from 231 lactating Black Bengal does of three semi-intensive goat farms in Bangladesh during the period from June to November 2009. Milk samples were collected by the farm attendants instructing them to soak the teat with 70% ethanol and drying off by tissue paper, one to two drops of milk were discarded and then 10 mL of milk were taken from each udder halves and kept in sterilized test tubes leveled with (L or R; L for left and R for right udder half). The following three tests were applied simultaneously on all milk samples collected for detection of mastitis.

California Mastitis Test (CMT): The reagent commercial CMT kit (Leukocyttest®, Synbiotic Corporation, France) used composed of Alkyl Aryl Sulfonate (3%), Sodium hydroxide (1.5%) and bromocresol purple (1:10,000) as an indicator. The test was performed and scored according manufacture's instruction with a slight modification described by Assefa *et al.* (2006). A doe was defined as cmt positive if it had at least one udder half with a cmt score of 1+ or above.

White Side Test (WST): Modified White Side Test was performed with a 4.0% solution of sodium hydroxide following the procedure described by Kahir (2006).

Surf Field Mastitis Test (SFMT): Surf Field Mastitis Test using 3.0% solution of household detergent (Surf Excel®, Uniliver, Bangladesh Ltd.) was performed and scored for screening of SCM in goats following the method described by Muhammad *et al.* (1995).

Statistical analysis: Integrated test result were interpreted based on the both "the OR rule" and "the AND rule" of parallel interpretation of clinical tests described by Weinstein *et al.* (2005). Calculation of confidence intervals for population proportions was performed by the Wilson score method without continuity correction (Newcombe, 1998).

RESULTS AND DISCUSSION

Prevalence based on individual test results: Prevalence of subclinical caprine mastitis in animal level was recorded as 39.83, 38.96 and 38.10% by CMT, WST and SFMT, respectively whereas 35.05, 34.85 and 31.60% by CMT, WST and SFMT, respectively in the level of udder halves (Table 1). These results agreed with the findings of earlier studies by several authors. Several authors reported the

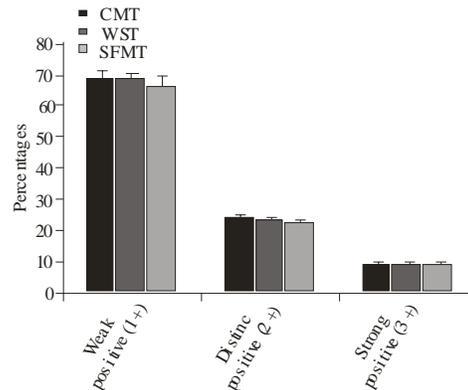


Fig. 1: Severity of reactions of three screening tests with mastitic milk of goats

prevalence of subclinical mastitis in dairy goat ranges between 19.4 and 47% (Contreras *et al.*, 2003), from 20 to 50% (Bergonier and Berthelot, 2003) and from 5 to 30% (Contreras *et al.*, 2007). This large variability of prevalence may be caused by different host and management risk factors that influence intra-mammary infection of goats.

Among the positive test results, 66.9, 67.7 and 66.44% samples showed weak positive (score 1+) reaction with CMT, WST and SFMT, respectively; 24.08, 22.98 and 22.6% showed distinct positive (score 2+) with CMT, WST and SFMT, respectively and 9.26, 9.26 and 9.59% showed strong positive (score 3+) reaction with CMT, WST and SFMT, respectively (Fig. 1). In this study, CMT score of 1+ was considered positive for mastitis and of trace and negative (\pm and 0) together was considered negative for subclinical mastitis. The CMT allows to roughly estimating the number of somatic cells in milk and positive reaction seems to depend on the concentration of somatic cells in the milk (Sharma *et al.*, 2008) thus CMT is influenced by factors causing variations in Somatic Cell Count (SCC). Unlike cow milk, which is produced by alveolar merocrine secretion, goat milk is produced by apocrine secretion and contains fairly high somatic cell content compare to cow milk (Wooding *et al.*, 1977). Despite possible differences between bovine milk and caprine and ovine milk, the CMT has been shown to be a reliable method for the diagnosis of subclinical mastitis in goats (Maisi and Riipinen, 1988). Sharma *et al.* (2008) reported SCC as the most accurate test for the diagnosis of subclinical mastitis followed by the Modified California Mastitis Test (MCMT) and the Modified White Side Test (MWST) while Patel *et al.* (2000) reported higher reliability of CMT (85.69%) followed by MWST (79.74%).

Among 462 milk samples screened by indirect tests, about 187 samples showed positive reaction with

Table 1: Animal wise and udder half wise prevalence of subclinical mastitis in goats in Bangladesh detected by three screening tests

Tests used	Types	Sample tested	Positive cases	Prevalence (95% CI*)
California mastitis test	Goat	231	92	39.83% (33.73-46.26%)
	Udder halves	462	162	35.05% (30.85-39.52%)
White side test	Goat	231	90	38.96% (32.90-45.38%)
	Udder halves	462	161	34.85% (30.64-39.30%)
Surf field mastitis test	Goat	231	88	38.10% (32.08-44.50%)
	Udder halves	462	146	31.60% (27.53-35.98%)

*CI: Confidence Interval

Table 2: Results of three screening tests in different combinations

CMT	WST	SFMT	Observations
+	+	+	187
+	+	-	18
+	-	+	1
+	-	-	2
-	+	+	0
-	+	-	0
-	-	+	0
-	-	-	254

-: negative test result; n: 462+; positive test result ; n: 462

all three tests and 254 samples showed negative reaction with all three tests. About 206 samples showed positive reaction with at least one of three tests and 275 samples showed negative reaction with at least one of three tests (Table 2).

Prevalence based on “the OR rule” of parallel interpretation: In term of parallel test interpretation, an animal is considered to have the disease, if one or more tests are positive performed on same animal. In particular “the OR rule” yield a positive diagnosis of mastitis if at least one test gives positive result. In this study, a total of 206 samples give positive results either CMT or WST or SFMT gives positive results yield a prevalence of 44.59 with 95% confidence interval of 40.12 to 49.15%, respectively.

Prevalence based on “the AND rule” of parallel interpretation: The assumption of “the OR rule” of parallel interpretation of multiple tests is that a positive diagnosis considered if all the tests used give positive result for the disease concerned. In this study, a total of 187 samples give positive results combine with CMT, WST and SFMT gives positive results yield a prevalence of 40.48 with 95% confidence interval of 36.10 to 45.01%, respectively.

Prevalence of subclinical mastitis in goats recorded in this study ranged from 31.60 to 35.05% in the level of udder half and from 38.10 to 39.83% in animal level when tests interpreted individually. But integrated test results yield the prevalence of caprine subclinical mastitis as 44.59% based on the OR rule and 40.48% based on “the AND rule” of parallel interpretation. It was also observed that combine tests results increases the likelihood of positive cases. Muhammad *et al.*

(2010) reported the sensitivity of SFMT, SCC, culture, CMT and WST in parallel testing as 72.82, 81.55, 87.38, 75.73 and 54.37%, respectively in cows, while 66.22, 79.73, 82.43, 70.27 and 50.0%, respectively in buffaloes. Among the three tests, reagents used in the SFMT can be found everywhere in the local areas and easily adoptable at the farm as compared to the reagents used in the WST and CMT. Reagents used in the WST can easily be found in big cities and therefore, it is the second easily available and adoptable test.

Parallel and serial interpretations are two important approaches for integration of information provided by the individual test results for diagnostic conclusion (Pfeiffer, 2002). Assumption of parallel interpretation is that tests are performed at the same time and interpreted together for a disease of interest whereas in series interpretation tests are performed one by one and the results of the first test determine whether the second test is performed at all (Zhou *et al.*, 2002). Though the serial interpretation of test result take the advantage of avoiding unnecessary tests, it causes potentially delaying treatment for diseased patients by lengthening the diagnostic testing period and thus not suitable for a common disease with rapid process towards it worse fate. Because of frequent occurrence of the subclinical form of mastitis which consequently causes clinical mastitis influenced by several stress factors early diagnosis and measures to be taken accordingly. Though bacteriological examination of milk is gold standard test for mastitis which is time consuming laborious process and not readily available at field condition, parallel interpretation of two or three screening test can be suggested for rapid assessment of caprine mastitis because animal is considered positive after first test since in that case the second test result does not matter anymore. If the first test is negative, the second test is still necessary. Parallel test interpretation based on “the OR rule” will increase sensitivity and the predictive value of a negative test result; therefore disease is less likely to be missed. Furthermore, Sensitivity and Specificity of all these tests for screening of caprine mastitis is recommended to be measured for choosing the best one or combinations in term of performance and cost effectiveness.

CONCLUSION

In this study, prevalence of subclinical mastitis in goats recorded as 44.59% based on the OR rule and 40.48% based on “the AND rule” of parallel interpretation whereas prevalence ranged from 31.60 to 35.05% in the level of udder half and from 38.10 to 39.83% in animal level when tests interpreted individually. CMT in combination with one or two other indirect low cost indirect tests can be recommended for screening of caprine mastitis in field condition with “the OR rule” of parallel interpretation.

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