Pakistan Vet. J., 2006, 26(2): 73-79.

COMPARATIVE EFFICIENCY OF SOME INDIRECT DIAGNOSTIC TESTS FOR THE DETECTION OF SUB-CLINICAL MASTITIS IN COWS AND BUFFALOES

M. IQBAL, M. AMJED[1], M. A. KHAN, M. S. QURESHI[1] AND U. SADIQUE[1]

Veterinary Research Institute, NWFP, Peshawar,
[1]NWFP Agricultural University, Peshawar, Pakistan

ABSTRACT

The present study was undertaken to compare five laboratory diagnostic tests for sub-clinical mastitis in cattle and buffaloes and to compute cost, time taken by each test and its ranking for availability, adoptability, interpretability and sensitivity. There were 352 cases with each test type viz. California Mastitis Test (CMT), White Side Test (WST), White Side + Dye (WSTD), Surf Test and Surf + Dye, and 880 cases with each species type (cattle and buffaloes). Result scores (1760) for sub-clinical mastitis in each category of negative, trace, single positive, double positive and triple positive by species, and laboratory tests, were analyzed using nonparametric tests. Chi-square statistics showed that CMT was equally effective at both locations (farm vs. laboratory). Correlation further suggested that the association was highly significant. Moreover, cases in category of negative, trace and single positive strongly differed ($P<0.05$) amongst the laboratory tests. But differences were found vague in double and triple positive categories. However, Chi-square statistics of overall sub-clinical mastitis cases showed that the laboratory tests were significantly different for detecting various categories of sub-clinical mastitis of a cross-tabulation. The present findings uncovered stronger ($P<0.05$) positive correlation of both the WST and WSTD with CMT, while of Surf Test and Surf + Dye with CMT. Results also suggested that WSTD and Surf + Dye were equally associated with CMT in strength and direction as their counterpart tests WST and Surf Test with CMT and thus its response in efficacy to added dye was not distinct. Species effect on result scores was found negligible ($P>0.05$). The study further suggested that CMT was the most sensitive test, followed by WST/WSTD and Surf/Surf + Dye. Although, the five tests showed slight discrepancy in the trace category reaction, a strong relationship of Surf Test to CMT, its low cost, easy availability and readily adoptable qualities should spur the relevant authorities to recommend the use of Surf test as a routine practice in dairy farming and add this test in the curriculum of diploma and degree programmes.

Key words: Diagnostic tests, sub-clinical mastitis, cows and buffaloes.

INTRODUCTION

Mastitis (inflammation of udder) is reorganized worldwide as the most important and costly disease of dairy animals. This condition is widespread in diary herds and accounts for enormous losses in milk yield and quality. These costs are borne directly by milk producers and indirectly by the consumers of dairy products.

Mastitis occurs in two forms i.e. clinical and sub-clinical. Clinical mastitis does not pose any problem in its detection, because of the grossly visible changes in the affected gland and its secretion. On the other hand, sub-clinical mastitis persists in the udder without causing any gross abnormality both in the gland and milk. Sub-clinical mastitis is only detected by testing milk samples. This form of the disease is important because it is 15 to 40 times more prevalent than its clinical counterpart and usually precedes the clinical form. Moreover, it is of long duration and is difficult to detect. It reduces milk production and adversely affects milk quality.

The present study was designed to investigate the comparative efficiency of five indirect tests for the diagnosis of sub-clinical mastitis in cattle and buffaloes. Results of this study will help to identify most appropriate test i.e. inexpensive, less time consuming, easily available, adoptable, interpretable and more sensitive for the diagnosis of sub-clinical mastitis under local farm conditions.

MATERIALS AND METHODS

Animals

Apparently healthy animals were selected to screen cattle and buffaloes (880 each) for sub-clinical mastitis by California Mastitis Test (CMT) at 26 dairy farms (public and private). Milk samples were collected twice from each animal at seven to ten days intervals and taken to the laboratory within two hours for further processing.

Collection of samples

The sample bottles (50 ml) were washed with tap water, air dried, plugged and sterilized at $160^{o}C$ in the hot air oven for one hour. The bottles were then labeled indicating the dairy farm, name or number of animal and collection date. Prior to collection of milk samples, the udder was washed with tap water and dried with paper towel. The quarters were disinfected with a pieces of cotton soaked in 70% ethyl alcohol. Mixed milk were collected from all four quarters after discarding a few streams and tested on spot by CMT (Schneider and Jasper, 1964). Representative milk samples collected in sterilized bottles (50 ml) were then shifted to the laboratory in a thermal flask containing crushed ice and subjected to five laboratory tests, as detailed below:

California Mastitis Test (CMT)

CMT kit used was supplied by Techni. Vet., Inc. USA. It is composed of Alkyl Aryl sulfonate (3%), sodium hydroxide (1.5%) and bromocresol purple (1:10,000) as an indicator. The test was conducted and scored according to Schneider and Jasper (1964). For conducting the CMT, a shallow half black paddle having four cups was used and was rinsed after each use. About 5 ml milk was drawn from bottle into the cup 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. The reaction developed almost immediately with milk containing a high concentration of somatic cells. The peak of reaction was obtained within 10 seconds and scored.

White Side Test (WST)

The WST reagent solution is composed of 4% sodium hydroxide. This test was performed and scored following the method described by Schalm and Gray (1954). For this purpose, a shallow half black paddle having four cups was used and was rinsed after each use. About 1 ml milk was drawn from bottle into the cup and an estimated five drops of WST reagent was squirted from a polyethylene wash bottle. Mixing was accomplished by gentle circular motion of the paddle in a horizontal plane. The reaction developed almost immediately with milk containing a high concentration of somatic cells. The peak of reaction was obtained within 30 seconds and immediately scored.

White Side + Dye Test (WSTD)

The reagent solution (4% sodium hydroxide) was modified with bromocresol (1:10,000) in the laboratory. The test was performed and interpreted following the same procedure as described above for WST. The peak of reaction was obtained within 30 seconds and immediately scored.

Surf Test

Reagent solution for surf test was composed of 3% household detergent (Surf, Lever Brothers Pakistan). This test was performed and scored following the method described by Rehman (1995) and Muhammad *et al.* (1995). A shallow half black paddle having four cups was used and was rinsed after each use. About 1 ml milk was drawn from bottle into the cup and an estimated 5 ml reagent was squirted from a polyethylene wash bottle. Mixing was accomplished by gentle circular motion of the paddle in a horizontal plane. The reaction developed almost immediately with milk containing a high concentration of somatic cells. The peak of reaction was obtained within 30 seconds and immediately scored.

Surf + Dye Test

The reagent solution of 3% household detergent (Surf) was modified with bromocresol (1:10,000) in the laboratory. The test was performed and interpreted following the same procedure as described above for Surf test. The peak of reaction was obtained within 30 seconds and immediately scored.

Scoring of milk samples

The five laboratory tests were compared for scoring milk samples (negative, trace, single positive, double positive and triple positive) by using grading procedure of CMT; (Schneider and Jasper, 1964) and WST (Schalm and Gray, 1954).

Statistics

The scores from the laboratory tests were recorded and compiled using the computer software programme (Microsoft Excel, 1998). Categorical data analysis procedure in statistical analysis system (SPSS Inc, 1998) was used for further statistics on the laboratory results. Nonparametric tests of significance (Levin and Fox, 1994) were used to analyze discrepancies of sub-clinical mastitis scores in each category of negative, trace, single positive, double positive and triple positive, obtained from various laboratory tests (CMT, WST, WSTD, Surf and Surf + Dye) for two species (cattle and buffaloes). Kruskal-Wallis, Jonckeeere-Terpstra and Medain Tests for one-way analysis of variance and Mann-Whitney U, Wald-Wolfowits and two samples Kolmogorov-Smirnov Statistics for two-way analysis of variance were used.

RESULTS AND DISCUSSION

California Mastitis Test as the control test

Soundness of CMT to screen cows and buffaloes at the farm was close to that in the laboratory. To confirm this, the same milk samples were subjected to CMT at both locations (farm and laboratory) and percent sub-clinical mastitis cases in each category of trace, single positive, double positive and triple positive. Chi-square statistics (Table 1) showed a significant association ($P<0.05$) between the two locations.

A non parametric statistics (Mann-Whitney U) equivalent to the t-test further confirmed the findings that CMT used at the farm, and when the same milk samples again tested in the laboratory, did not change its effectiveness. Correlations coefficient (Kendall's tau_b and Spearman's rho) also indicated stronger relationship ($P<0.05$) with large absolute values (r = 0.98; P = 0.98) near to one (Table 2). CMT was also used by Tekeli *et al*. (1992) as a control test with electric conductivity measurement of milk for mastitis detection.

Table 1: Chi-square statistics of sub-clinical mastitis cases diagnosed by California Mastitis Test at farm and laboratory

	Chi-square value	Df	Level of significance
Pearson Chi-square	999.000a	9	0.000
Likelihood ratio	753.122	9	0.000
Linear-by-linear association	336.173	1	0.000
Number of valid cases	352		

a=3 cells (18.8%) have expected count less than 5. The minimum expected count is 1.25.

Mann-Whitney ranks and test statistics of sub-clinical mastitis cases in each category of trace, single positive, double positive, and triple positive for CMT at farm versus laboratory are shown in Table 3. Larger significance values ($P>0.05$) indicated that the results at two locations were similar.

These findings revealed that the sensitivity of the CMT was not affected when the milk samples were collected and brought to the laboratory for use as control test for other tests of sub-clinical mastitis. Sub-clinical mastitis cases in each category (trace, single positive, double positive, and triple positive) as affected by two species (cattle and buffalo) and locations (laboratory versus farm) are also shown in Table 4 (count and expected count). The data strongly indicate that proportion of sub-clinical mastitis in each category of trace, single positive, double positive and triple positive were not different due to two species (cattle versus buffaloes) and locations (laboratory and farm).

Efficiency of the laboratory tests

Numbers (observed and expected) and Chi square statistics (Tables 5 to 9) were computed for sub-clinical mastitis cases by tests, namely CMT, WST, WSTD, Surf and Surf + Dye for each category separately. The key point to note in Table 10 is that CMT is not shown for negative cases because milk samples were initially collected to screen cows and buffaloes for sub-clinical mastitis. Small significance level ($P<0.05$) indicates that cases in each category of negative, trace and single positive strongly differed among the laboratory tests (WST, WSTD, Surf, Surf + Dye and CMT). However, differences were found vague in double and triple positive categories of sub-clinical mastitis among these laboratory tests (Tables 8 and 9).

Results of the present study reveal that the laboratory tests are nearly equally effective as the severity of sub-clinical mastitis increases in milking animals. The sensitivity of various laboratory tests is open to interpretation and conflicts when they are used for milking animals having a low level of severity of sub-clinical mastitis. The sensitivity is also questionable for negative cases as those shown with WST, WSTD, Surf and Surf + Dye but not with CMT. Other studies (Rehman, 1995; Mohammad *et al.*, 1995) also recorded such discrepancies at the border line of each category of sub-clinical mastitis cases. It is further pointed out that added dye (WSTD and Surf + Dye) had not picked up any better outcome effect to their counterpart tests (WST and Surf test). Didonat *et al.* (1986) also reported that the bromocresole purple test was less responsive to the sub-clinical mastitis in buffaloes than modified WST and CMT.

Table 11 shows Chi-square statistics of overall sub-clinical mastitis cases in each category of negative, trace, single positive, double positive and triple positive by various laboratory tests. These results showed that the laboratory tests were significantly different for detecting various categories of sub-clinical mastitis of a cross-tabulation (Table 10).

Table 2: Nonparametric correlations of California Mastitis Test for sub-clinical mastitis cases at farm (SCMT) and laboratory (LCMT) (n=352)

Correlations				
			SCMT (Screening Detergent Test)	**LCMT** (Lab Detergent Test)
Kendall's tau_b	SCMT (Screening Detergent Test)	Correlation coefficient	1.000	0.978**
	LCMT (Lab Detergent Test)	Correlation coefficient	0.978**	1.000
Spearman's rho	SCMT (Screening Detergent Test)	Correlation coefficient	1.000	0.980**
	LCMT(Lab Detergent Test)	Correlation coefficient	0.980**	1.000

**Correlation is significant at the 0.01 level (2-tailed).
b. Assuming data distribution is nonparametric.

Table 3: Mann-Whitney ranks and test statistics of sub-clinical mastitis cases in each category of trace, single positive, double positive and triple positive by California Mastitis Test (CMT) at farm (SCMT) and laboratory (LCMT)

Ranks				
Sub-clinical mastitis	**CMT**	***N**	**Mean rank**	**Sum of ranks**
Trace	SCMT (Screening Detergent Test)	185	186.50	34502.50
	LCMT (Lab Detergent Test)	187	186.50	34875.50
	Total	372		
Single positive	SCMT (Screening Detergent Test)	91	91.00	8281.00
	LCMT (Lab Detergent Test)	90	91.00	8190.00
	Total	181		
Double positive	SCMT (Screening Detergent Test)	54	55.00	2970.00
	LCMT (Lab Detergent Test)	55	55.00	3025.00
	Total	109		
Triple positive	SCMT (Screening Detergent Test)	22	21.50	473.00
	LCMT (Lab Detergent Test)	20	21.50	430.00
	Total	42		

*N stands for number of cases.

Table 4: Number of sub-clinical mastitis cases in each category of trace, single positive, double positive, triple positive by California Mastitis Test at farm (SCMT) versus laboratory (LCMS) for two species

Species	**Results**		**SCMT** (Screening Detergent Test)	**LCMT** (Lab Detergent Test)	**Total**
Cattle	Trace	Count	90.0	92.0	182.0
		Expected count	91.0	91.0	182.0
	Single positive	Count	42.0	41.0	83.0
		Expected count	41.5	41.5	83.0
	Double positive	Count	34.0	35.0	69.0
		Expected count	34.5	34.5	69.0
	Triple positive	Count	10.0	8.0	18.0
		Expected count	9.0	9.0	18.0
	Total	Count	176.0	176.0	352.0
		Expected count	176.0	176.0	352.0
Buffalo	Trace	Count	95.0	95.0	190.0
		Expected count	95.0	95.0	190.0
	Single positive	Count	49.0	49.0	98.0
		Expected count	49.0	49.0	98.0
	Double positive	Count	20.0	20.0	40.0
		Expected count	20.0	20.0	40.0
	Triple positive	Count	12.0	12.0	24.0
		Expected count	12.0	12.0	24.0
	Total	Count	176.0	176.0	352.0
		Expected count	176.0	176.0	352.0

Table 12 displays the number of cases for each level of each factor (laboratory tests and species). There are 352 cases with each test type (WST, WSTD, Surf, Surf + Dye and CMT) and 880 cases with each species type (cattle and buffaloes). Tests of between-subject effects in Table 12 showed that laboratory tests had significant ($P<0.05$) effect on number of sub-clinical mastitis cases but the species effect was found not significant.

Table 13 displays the cost and time taken by sub-clinical mastitis tests and their ranking for availability, adoptability, interpretability and sensitivity. Costs and

Table 5: Numbers (observed and expected) and Chi-square statistics of negative cases obtained from laboratory sub-clinical mastitis tests

	Number of cases		
Lab tests	Observed	Expected	Residual
White Side test	115	141.8	-26.8
White Side + Dye test	117	141.8	-24.8
Surf test	168	141.8	26.3
Surf + Dye test	167	141.8	25.3
Total	567	-	-

Table 6: Numbers (observed and expected) and Chi-square statistics of trace cases obtained from laboratory sub-clinical mastitis tests

	Number of cases		
Lab tests	Observed	Expected	Residual
White Side test	115	118.0	-3.0
White Side + Dye test	113	118.0	-5.0
Surf test	87	118.0	-31.0
Surf + Dye test	88	118.0	-30.0
California Mastitis test	187	118.0	69.0
Total	590	-	-

Table 7: Numbers (observed and expected) and Chi-square statistics of single positive cases from laboratory sub-clinical mastitis tests

	Number of cases		
Lab tests	Observed	Expected	Residual
White Side test	51	56.4	-5.4
White Side + Dye test	53	56.4	-3.4
Surf test	44	56.4	-12.4
Surf + Dye test	44	56.4	-12.4
California Mastitis test	90	56.4	33.6
Total	282	-	-

Table 8: Numbers (observed and expected) and Chi-square statistics of double positive cases from laboratory sub-clinical mastitis tests

	Number of cases		
Lab tests	Observed	Expected	Residual
White Side test	50	44.0	6.0
White Side + Dye test	49	44.0	5.0
Surf test	33	44.0	-11.0
Surf + Dye test	33	44.0	-11.0
California Mastitis test	55	44.0	11.0
Total	220	-	-

Table 9: Numbers (observed and expected) and Chi-square statistics of triple positive cases from laboratory sub-clinical mastitis tests

	Number of cases		
Lab tests	Observed	Expected	Residual
White Side test	21	20.2	0.8
White Side + Dye test	20	20.2	-0.2
Surf test	20	20.2	-0.2
Surf + Dye test	20	20.2	-0.2
California Mastitis test	20	20.2	-0.2
Total	101	-	-

ranking are computed based on the authors self observations and not really the outcome of study survey from farmers interviews. The cost per CMT was Rs. 3.0 as compared to Rs. 0.45 and Rs. 0.40 for WST and Surf test (in case of added dye Rs. 0.05 increased for WSTD and Surf + Dye). This is because the reagents used in CMT are imported from abroad. However, time taken for preparing reagents and carrying out CMT is less (10 seconds) than that of the other individual indigenous tests (30 seconds). Among the five tests, reagents used in the Surf Test 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. The tests incorporating dye component are similar in ranking with WST with regard to availability and adoptability.

California Mastitis Test and the other two tests (WSTD and Surf + Dye) with added dye components

Table 10: Numbers (observed and expected counts) of sub-clinical mastitis cases by tests

Tests	Lab results					Total
	Negative	Trace	Single positive	Double positive	Triple positive	
WST	116	115	51	50	20	352
	(113.6)	(118.0)	(56.4)	(44.0)	(20.0)	(352.0)
WSTD	117	113	53	49	20	352
	(113.6)	(118.0)	(56.4)	(44.0)	(20.0)	(352.0)
Surf	168	87	44	33	20	352
	(113.6)	(118.0)	(56.4)	(44.0)	(20.0)	(352.0)
Surf + Dye	167	88	44	33	20	352
	(113.6)	(118.0)	(56.4)	(44.0)	(20.0)	(352.0)
CMT	0	187	90	55	20	352
	(113.60	(118.0)	956.4)	(44.0)	(20.0)	(352.0)
Total	568	590	282	220	100	1760
	(568.0)	(590.0)	(282.0)	(220.0)	(100.0)	(1760.0)

*Values in parentheses are expected counts.

Table 11: Cross-tabs Chi-squared statistics and symmetrical measures for relationship between sub-clinical mastitis cases and tests

Chi-square tests			
	Value	Df	Level of significance
Pearson Chi-square	257.140a	16	0.000
Likelihood ratio	357.216	16	0.000
Linear-by-Linear association	11.359	1	0.001
Number of valid cases	1760		

a=0 cells have expected count less than 5. The minimum expected count is 20.00

were easier to interpret the results than those which had no dye. Table 13 shows that the most sensitive test was CMT, followed by WST/WSTD and Surf/Surf + Dye.

Conclusions

The present findings suggested close relationship between CMT at farm and laboratory to screen out cows and buffaloes for sub-clinical mastitis. Efficiency of CMT was better than that of other four tests at the border line of negative and trace, when the inflammation in the udder was at subclinical stage. Discrepancy in test scores was not found due to species effect. Although the indigenous tests were not as sensitive as CMT, this factor was counter balanced by the benefit of low cost, easy availability and adoptability qualities of other tests.

REFERENCES

Didonat. N., P. Singh and H. H. Justiniavi, 1986. Comparison of indirect tests for diagnosis of sub-clinical mastitis in buffaloes. Bulletin De Pequisa Centro De Pequisa Agropecuaria do Emtorapa, Brazil, 77: 13-17.

Levin, J. and J. A. Fox, 1994. Elementary Statistics in Social Research. Harper Collins College Publishers, New York, USA, pp: 353.

Muhammad, G., M. Athar, A. Shakoor, M. Z. Khan, F. Rehman and T. Ahmad, 1995. Surf field mastitis test: an inexpensive new tool for evaluation of wholesomeness of fresh milk. Pak. J. Food Sci., 5(3-4): 91-93.

Newbould, F. H. S., R. S. Butler and S. D. Scres, 1982. “Mastitis of dairy cows” video views. Fact sheet No. 8. Veterinary Infectious Diseases Organization, Sackotoon, Saskatchewan, Canada.

Table 12: GLM univariate and test between subject’s factors for number of cases and Analysis of Variance at each level of laboratory tests and species

Source	Sum of squares	Df	Mean squares	F-value	Probability level
Model	9101.299a	6	1516.883	1115.701	0.000
Test	125.712	4	31.428	23.116	0.000
Species	2.475	1	2.475	1.820	0.117
Error	2384.701	1754	1.360	-	-
Total	11486.000	1760	-	-	-

Table 13: Cost and time taken, sub-clinical mastitis tests and their ranking for availability, adoptability, interpretability and sensitivity

	Sub-clinical mastitis tests				
Parameters	**WST**	**WSTD**	**Surf**	**Surf+Dye**	**CMT**
Cost per test (Rs.)[a]	0.45	0.50	0.40	0.45	3.00
Time taken per test (sec)[b]	30.0	30.0	30.0	30.0	10.0
Availability [c]	2	2	1	2	3
Adoptability [d]	2	2	1	2	3
Interpretability[e]	2	1	2	1	1
Sensitivity [f]	2	2	3	3	1

a. Cost per test includes test ingredient (s).
b. Time taken per test measured from time required for preparing reagent and test conducted.
c. Availability observed on the ease of access to the test reagent (s) in the study area.
d. Adoptability observed on the ease of use of various laboratory sub-clinical mastitis tests.
e. Interpretability based on the degree of appearing the gel consistency.
f. Sensitivity based on negative/positive cases of sub-clinical mastitis.

Rehman, F., 1995. Studies on: (I) evaluation of surf field mastitis test for the detection of sub-clinical mastitis in buffaloes and cattle, and (II) antibiotic susceptibility of the pathogens. MSc Thesis, Univ. Agri., Faisalabad, Pakistan.

Schalm, O. W. and D. Gray, 1954. The White Side test for detection of mastitic milk. Calif. Vet., 7: 27.

Schneider, R. and D. E. Jasper, 1964. Standardization of the California Mastitis Test. Am. J. Vet. Res., 25: 1635.

Tekeli, T., A. Semacan and K. Isik, 1992. Detection of sub-clinical mastitis with a handy instrument by measuring the electrical conductivity of milk. Livestock Advisor (India), 17(11): 7-30.