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COMPARISON OF SOME INDIRECT SCREENING TESTS FOR DETECTION OF SUBCLINICAL MASTITIS IN DAIRY COWS

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Summary

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A total of 335 quarter milk samples from crossbred dairy cows were subjected to California mastitis (CMT), sodium lauryl sulphate (SLST), and somatic cell count (SCC) tests. CMT positive milk samples were 227, while 108 samples were CMT negative. Milk samples positive by SLST and SCC were 261 and 180 respectively. The CMT positive samples were subjected to cultural isolation (gold standard test for comparison of indirect mastitis tests). Of these, 201 samples were positive by cultural isolation. The sensitivity of the CMT, SLST and SCC was 86.07%, 74.63% and 88.60%; specificity 59.70%, 17.16% and 97.76%; percentage accuracy 75.52%, 51.64% and 91.94%; positive predictive value 76.21%, 57.47% and 98.33%; and negative predictive value - 74.07%, 31.08% and 84.52%, respectively. Kappa value of SCC was higher than that of CMT. CMT was concluded to be the most accurate test after cultural isolation and SCC. Unlike laboratory tests as cultural isolation and SCC that require adequate laboratory facilities and skilled personnel, CMT is a reliable diagnostic method in field conditions.

Key words: bacteriological culture, California mastitis test, mastitis, milk, sodium lauryl sulphate test, somatic cell count

INTRODUCTION

Mastitis is the most costly disease of dairy cattle due to economic losses from reduced milk production, treatment costs, increased labour, milk withheld following treatment, death, and premature culling (Kaneene & Hurd, 1990; Miller *et al.*, 1993). Early detection of mastitic cows is important for most dairy farmers to reduce production losses and to enhance prospects of recovery. Diagnosis of clinical mastitis is based on the local and systemic reactions and changes in milk (e.g. offcolor, watery, bloody appearance and presence of flakes, clots and pus). The diagnosis of subclinical mastitis is more problematic since the milk appears normal but usually has an elevated somatic cell count. Early diagnosis of mastitis is vital because changes in the udder tissue take place much earlier than they become apparent.

Various methods, based on physical and chemical changes of milk and cultural isolation of organisms, are used for diagnosis of subclinical mastitis (Batra & Mcallister, 1984; Emanuelson *et al.*, 1987). The diagnosis of mastitis according to the International Dairy Federation (IDF) recommendations is based on the

somatic cell counts (SCC) and microbiological status of the quarter. Bacteriological culture of milk samples is the standard method for identifying mastitis. However, the logistic and financial considerations involved with sampling all fresh cows have precluded this technique from being widely adopted. Milk culture identifies the presence of mastitis pathogens but does not provide a measure of the degree of inflammation associated with the infection.

Among the simplest tests, which do not require any complex laboratory equipment, are solutions containing detergents. Detergents decrease surface tension, change the structure and conductivity of cell membrane and nucleus, disturb osmotic balance, block oxidizing and stimulate proteolytic enzymes, and increase milk viscosity (Sargeant *et al.*, 2001; Ruegg & Reinemann, 2002; Middleton *et al.*, 2004). The California mastitis test (CMT) and sodium lauryl sulphate test (SLST) are arguably the only reliable cowside screening tests for subclinical mastitis that can easily be applied. The CMT is a simple, inexpensive, rapid screening test for subclinical mastitis, based upon the amount of cellular nuclear protein present in the milk sample. Modern mastitis tests allow for indirect determination of the number of somatic cells in milk (Greiner *et al.*, 2000). As the inflammatory process develops in udder tissue, the number of these cells (particularly leukocytes) in milk sharply increases. SCC, CMT and intramammary infection are associated significantly; therefore these parameters provide the necessary information to evaluate udder health status in cows.

The objective of the present study was to evaluate and compare three indirect mastitis diagnostic tests – California mastitis test (CMT), sodium lauryl sulphate test (SLST) and somatic cell count (SCC) for their ability to classify correctly udder health status of individual cows.

MATERIALS AND METHODS

Animals

The present study was carried out on organized (well managed) dairy farm, Jammu, India. A total of 85 crossbred dairy cows (Holstein-Friesian × Jersey) of different age groups (3 to 11 years), parities (1 to 7) and stage of lactation (early, mid and late) were included in the study group. All cows were housed in tie stall barns and milked twice daily by hand. The regular practices of washing of udder before milking with potassium permanganate solution were used by milkers.

Sample collection

Milk samples (n=335) were collected after proper disinfection of teat surface with 70% ethanol. Sixty mL of milk from all four quarters were collected aseptically in separate sterile polyethylene screw-capped, wide mouth vials after squirting few streams (Buswell, 1995). CMT was conducted at the spot before milk sample collection. The milk samples were placed in an ice box and carried to the college laboratory, where they were kept at 4 to 10°C in a refrigerator for further laboratory investigation.

Diagnostic tests

The milk samples from apparently healthy cows were subjected to following diagnostic tests: California mastitis test (CMT), sodium lauryl sulphate test (SLST) and somatic cell count (SCC). The CMT were performed cow-side and according to manufacturer's recommendations on quarter foremilk samples. In

brief, 3 mL of milk sample was taken in the CMT paddle and equal quantity of CMT reagent (California Mastitis Kit of M/s B.V. Bio-Corp Pvt Ltd, Pune, India) was added in each cup, rotated for few seconds and the result was recorded within 30 s as 0 (negative), T (trace), 1+, 2++, or 3+++. SLST (Pandit & Mehta, 1969) procedure was the same as for CMT: 3 mL of milk sample was taken in the CMT paddle and equal quantity of sodium lauryl sulphate (3% solution in distilled water, pH adjusted to 7.0) was added in each cup, rotated for few seconds and the result was recorded within 30 s as 0 (negative), T (trace), 1+, 2++, or 3+++. The SCC test was performed according to Schalm *et al.*, (1971).

Animals were considered positive for mastitis when CMT and SLST score was ≥1+ and SCC value was $\geq 2\times10^5$/mL of milk (threshold value).

CMT positive samples (n=227) were subjected to bacteriological culture as per Quinn *et al.* (1994).

The following diagnostic test characteristics were determined using the milk bacteriological culture result as a gold standard control.

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

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

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

$$\text{Positive predictive value} = \frac{TP}{TP + FP} \times 100$$

$$\text{Negative predictive value} = \frac{TN}{FN + TN} \times 100$$

where: TP – true positive, FP – false positive, TN – true negative, FN – false negative.

Statistical analysis

The percentage accuracy of the tests and sensitivity, specificity, and the predictive values of the CMT, SLST and SCC results, compared to culture results, were calculated using standard two-by-two contingency tables. Correlations between the dependent variables were calculated using Pearson's correlation.

RESULTS

Two hundred and twenty seven milk samples were CMT positive and 108 samples were CMT negative. Milk samples positive by SLST and SCC were 261 and 180 respectively. The accuracy of CMT, SLST and SCC was 75.52%, 51.64% and 91.94% (Table 1).

Table 1. Percentage accuracy of various indirect tests used for the diagnosis of mastitis. Data are presented as number (percentage).

Tests	Samples examined	Positive samples	TP	FP	TN	FN	Accuracy
CMT	335	227 (67.76)	173 (76.21)	54 (23.79)	80 (74.07)	28 (25.72)	75.52%
SLST	335	261 (77.91)	159 (57.47)	111 (42.53)	23 (31.08)	51 (68.92)	51.64%
SCC	335	180 (53.73)	177 (98.33)	3 (1.63)	131 (84.52)	24 (15.48)	91.94%

TP=true positive, FP=false positive, TN=true negative, FN=false negative.

Table 2. Agreement and correlation of various indirect tests used for the diagnosis of mastitis with bacteriological (cultural) examination

Test	Sensitivity, %	Specificity, %	PPV, %	NPV, %	PLR	NLR	κ	r, %	P
CMT	86.07	59.70	76.21	74.07	2.13	0.23	0.47	47.97	0.00
SLST	74.63	17.16	57.47	31.08	0.90	1.48	0.09	9.69	0.08
SCC	88.60	97.76	98.33	84.52	39.55	0.12	0.84	84.32	0.00

PPV=positive predictive value, NPV=negative predictive value, PLR=positive likelihood ratio, NLR=negative likelihood ratio, κ=Kappa (measure of agreement), r=Pearson's correlation.

The positive and negative predictive values and likelihood ratios are given in Table 2. In the present investigation, the sensitivity and specificity of the SCC were higher than those of CMT and SLST. The specificity of SLST was very low (17.16%). SCC showed the highest correlation (r=0.843) with bacterial culture test, followed by CMT and SLST. It is accepted that Kappa values <0.4 indicate poor agreement, values between 0.4 and 0.75: fair to good agreement, and values >0.75: excellent agreement. Thus, SCC exhibited excellent agreement with bacterial culture test while CMT had fair to good agreement (Table 2).

DISCUSSION

In the present study it was apparent that SCC was the most reliable test and closest to the bacteriological results. However, SCC is found out to increase in the first few days of lactation and may be high up to the first month of lactation (Atakan, 2008) and increase towards the end of lactation is considered to be physiological. Sederevicius *et al.* (2006) reported a temporary increase in SCC just after calving due to adaptation of the udder from non-lactating to lactating status, while in mid lactations SCC usually remains in normal range. High SCC in milk reduces the quality of both milk and dairy products, and also affects milk shelf life and flavour, as well as cheese and butterfat yield. The SCC increased above the normal range in case of inflammation or udder infection. The positive reaction of CMT and SLST seems to depend on the concentration of somatic cells in the milk (Sharma *et al*., 2008). SCC needs a skilled personnel, adequate laboratory facilities and time, just as bacterial culture, so other tests (e.g. CMT, SLST) were developed as alternatives.

In the present investigation, in regard to accuracy CMT was at 3rd position but from field point of view it is assessed as sufficient to diagnose the pre-clinical cases of mastitis. The principle of CMT and SLST is that the reagent (detergent) dissolves or disrupts the outer cell wall and the nuclear cell wall of leukocyte (somatic cells), which are primarily fat (detergent dissolves fat). DNA is released from the nuclei of somatic cells and it strings or gels together to form a stringy mass. As the number of leukocytes in a quarter increases, the amount of formed gel increases paralelly linearly. Of 227 CMT positive samples, subjected to cultural isolation, 201 samples were positive by cultural isolation.

Present findings are in agreement with Sharma *et al.* (2008). They reported that

SCC was 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). Patel *et al.* (2000) reported higher reliability of CMT (85.69%) followed by MWST (79.74%). Reddy *et al.* (1998) compared the specificity and sensitivity of CMT and SCC with standard cultural test and observed 100% predictive value with the cultural test of the milk, 84.84% specificity for SCC and 73.30% for CMT. Tanwar *et al.* (2001) also compared various diagnostic tests for detection of subclinical mastitis and indicated 100% sensitivity for SCC and 96% for CMT reaction. According to Goswami *et al.* (2003), animal-wise efficacy of indirect tests taking cultural examination as a standard was in the following descending order: SCC (97.46%), MCMT (69.62%) and MWST (63.29%). So, the present findings regarding accuracy and sensitivity of indirect tests for detection of subclinical mastitis support the earlier observations. From a study of SCC and CMT sensitivity and specificity for identifying intramammary infection in early lactation Sargeant *et al.* (2001) concluded that CMT could be used in dairy herd monitoring programmes as a screening test to detect fresh cows with intramammary infection caused by major pathogens. Barbosa *et al.* (2002) reported that the SCC and CMT are dependent and were highly correlated for diagnosis of subclinical mastitis. Sudhan *et al*. (2005) reported that the percentage of agreement of CMT, SLST, bromothymol blue card test and White side test with bacteriological examination were 57.89%, 62.07%, 64.46% and 68.65% respectively. The present study suggests that CMT could be used as regular mastitis screening test in field condition, even by less trained dairymen.

In conclusion, SCC was the most accurate test after cultural isolation, followed by CMT and SLST. Taking into consideration that laboratory tests such as cultural isolation and SCC require adequate laboratory facilities, personnel and time, CMT is a reliable diagnostic method for use in field conditions**.**

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