COMPARISON OF DIFFERENT DIAGNOSTIC TESTS FOR THE DETECTION OF SUBCLINICAL MASTITIS IN BUFFALOES

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ABSTRACT

Mastitis is common in buffaloes with significant impact on the rural economy. The present work was undertaken to find out the prevalence of sub clinical mastitis in buffaloes in and around Gannavaram, Andhra Pradesh, India by using different diagnostic methods. The prevalence of sub clinical mastitis based on California Mastitis Test, somatic cell count, electrical resistance and milk pH was 20.34, 21.62, 6.20 and 8.27%, respectively on screening of 629 quarters of 158 Graded Murrah buffaloes. Bacterial culture was considered to be a gold standard and revealed the prevalence as 28.30% with 178 quarter milk samples positive for bacterial growth. Further the study was also focussed on the comparison of the diagnostic tests CMT, SCC, ER and pH by using culture results as gold standard. The percent accuracy of California Mastitis Test, Somatic cell count, electrical resistance and pH were 89.83, 92.69, 77.27 and 79.65, respectively.

Keywords: *Bubalus bubalis*, buffaloes, sub clinical mastitis, diagnosis, culture, comparison

INTRODUCTION

India continues to be the largest producer of milk in the world with 190 million cattle and 108 million buffaloes as per 19th Livestock Census (2012). Of the total milk production, buffaloes contribute about 53% while cows have a 43% share. Mastitis has remarkably rising impact on Indian economy where overall losses due to mastitis is estimated to be Rs. 7165.51 crores of which 70% was due to subclinical mastitis (Bansal and Gupta, 2009). Subclinical mastitis is considered as a prevailing disease in dairy herds with prevalence of 15 to 40 subclinical mastitis cases for every one case of clinical mastitis (Kelly, 2002).Subclinical mastitis is an inflammation of the mammary gland without noticeable signs, with 15 to 45% reduction in daily milk yield and altered milk composition (Swinkels *et al.*, 2005; Halasa *et al.*, 2007).

Early diagnosis of mastitis is important for reducing production losses and for enhancing the prospects of recovery. There are several direct and indirect tests with varying efficacies for detection of subclinical mastitis. The sensitivity and specificity of the tests varies widely. Among the tests, bacterial culture from the milk has been

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considered as standard method for confirming subclinical udder infections in dairy cows (IDF, 2005; Sudhan and Sharma, 2010. But bacteriological examination of milk samples is time consuming and laborious. Hence the present study was instituted for comparing the sensitivity, specificity, and accuracy of other mastitis markers named California mastitis test, (CMT), somatic cell count (SCC), Electrical resistance (ER) and pH and to evaluate the efficacy of these tests by comparing with the results of the reference test.

MATERIALS AND METHODS

Sample collection

A total of 629 milk samples were collected aseptically from 158 buffaloes in and around Gannavaram and were subjected to the following diagnostic tests

California mastitis test (CMT)

A plastic paddle with four chambers of shallow cups was used to perform the test. About 3.0 ml of milk sample from each quarter was stripped directly into the cups. To this equal volume of California mastitis test reagent was added and mixed through gentle swirling the paddle in a circular motion for few seconds. Based on the thickness of the gel formed by CMT reagent-milk mixture, test results were scored as 0 (negative/ trace), +1 (weak positive), +2 (distinct positive), and +3 (strong positive) (Guha *et al*., 2012).

Somatic Cell Count (SCC)

The somatic cell count in milk samples was estimated as described by Kavitha *et al*. (2011).

Procedure

Milk samples were mixed 15 to 25 times to obtain a uniform distribution of cells. The samples were allowed to stand for 2 to 5 minutes to permit the air bubbles to rise and foam to disappear. Identification number of the sample was written on a clean microscopic slide. A level surface was selected and the slide was placed over the template to outline four 1 sq.cm areas.

Ten µL of milk was placed exactly in the centre of the 1 sq.cm template and was spread evenly to cover all the area delineated by the template. From each sample two films were prepared using successive areas of the slide. The films were dried at room temperature.

Newman-Lampert staining of milk samples

The slides with milk smears were placed on the slide rack and the smears were flooded with modified Newman-Lampert stain (Himedia) for 2 minutes. The excess stain was drained off by standing the slides on absorbent paper and then air-dried. The slide was rinsed in three changes of tap water at 42 to 45ºC and subsequently air-dried.

Counting of cells

Stained films were examined under oil immersion objective and the number of cells in 20 fields was counted. The fields were selected by moving the slide horizontally from one edge of the film through the centre to the opposite edge and then repeated in a vertical direction. The average number of cells per field was multiplied by the microscopic factor.

Cell count per ml of milk = Average number of cells per field × Multiplication factor × 100 (MF=5000).

In the present study the SCC of more than

2,00,000 per ml of milk was considered as positive for subclinical mastitis (Pamela, 2002; IDF, 2005; Guha *et al*., 2012).

Electrical resistance (ER)

Draminski Mastitis detector measures electrical resistance of milk to detect subclinical mastitis. A reading below 300 units was considered as the cut-off value for subclinical mastitis and above 300 was considered as healthy (Siddiquee *et al*., 2013).

pH

The pH of each milk sample was estimated by using narrow range pH Papers. Milk samples with pH more than 6.8 were considered positive for subclinical mastitis (Sharma *et al*., 2010).

Bacteriological culture of milk samples

All the milk samples were subjected for bacteriological examination. The culture and isolation of bacteria up to genus level was performed by employing standard procedures as described by Guha *et al.* (2012).

The percentage accuracy of the tests and sensitivity, specificity, and the predictive values of the CMT, SCC, ER and pH were determined using culture results as gold standard.

RESULTS AND DISCUSSION

The quarter wise prevalence of subclinical mastitis based on California Mastitis Test was 20.34% (128/629), somatic cell count 21.62% (136/629), while the same was 6.20% (39/629) and 8.27% (52/629) based on electrical resistance and milk pH. Bacterial culture revealed the prevalence of subclinical mastitis as 28.30% with milk samples of 178 quarters positive for bacterial growth. The percent accuracy of California Mastitis Test, Somatic cell count, Electrical resistance and pH were 89.83, 92.67, 77.27 and 81.08%, respectively. High accuracy of California Mastitis Test and somatic cell count than electrical resistance and pH might be due to quick immune response to foreign agents by the immune cells than alteration in the ionic concentration in the milk.

The percent true positive, true negative, false positive and false negative with all the tests are presented in Table 1. The incidence of false positive reactions was more in CMT which was in agreement with the findings of Kamal *et al*. (2014) who recorded false positive reactions in early or late lactation due to destruction of leukocytes by microbial toxins. False negative reactions were more in ER followed by pH, CMT and SCC. High sensitivity was observed with SCC (75.28%) followed by CMT (67.98%) was in agreement with the observations of Reddy *et al*. (2001); Tanwar *et al*. (2001) while specificity was more with pH (99.78) followed by ER (99.56), SCC (99.56) and CMT (98.45). Although specificity was more with pH, all the other tests also showed almost equal specificity. Based on kappa value, it was observed that all the tests were in perfect agreement with bacteriological culture. But in the present study estimation of somatic cell count was considered superior to other tests and was closer to culture examination results based on accuracy, high sensitivity, specificity, and positive predictive value followed by California Mastitis Test. High agreement of SCC was similar to that reported by Guha *et al.* (2012).

Table 1. Evaluation of CMT, SCC, ER and pH taking cultural examination as standard.

S. No.	Name of the diagnostic test	Total samples examined	Test positive samples	Test results as compared to cultural examination				Percentage accuracy
				True positive	False positive	True negative	False negative	
1	California Mastitis Test (CMT)	629	128	121 (94.53)	7 (5.47%)	444 (88.62)	57 (11.37)	89.83
2	Somatic Cell Count (SCC)	629	136	134 (98.53)	2 (1.49%)	449 (91.08)	44 (8.92)	92.69
3	Electrical Resistance (ER)	629	39	37 (94.87)	2 (5.41%)	449 (76.10)	141 (23.90)	77.27
4	pH	629	52	51 (98.08)	1 (1.96%)	450 (77.99)	127 (22.01)	79.65
5	Bacterial culture	629	178	178 (100.0)	_	451 (100.0)	_	100.00

Values in parenthesis indicate percentage

$$\text{Percentage of accuracy} = \frac{\text{True positive} + \text{True negative}}{\text{Number of samples examined}} \times 100$$

Table 2. Sensitivity, Specificity and Predictive value of different diagnostic tests on the basis of cultural examination as standard.

S. No.	Name of the test	Sensitivity	Specificity	Predictive value of positive test	Predictive value of negative test
1	California Mastitis Test (CMT)	67.98	98.45	94.53	88.62
2	Somatic Cell Count (SCC)	75.28	99.56	98.52	91.08
3	Electrical resistance	20.79	99.56	97.87	76.10
4	pH	28.65	99.78	98.08	77.98
5	Bacterial culture	100.00	100.00	100.00	100.00

1. Sensitivity = $\frac{\text{True positive}}{\text{True Positive + False negative}} \times 100$

2. Positive Predictive value = $\frac{\text{True positive}}{\text{True positive + False positive}} \times 100$

3. Specificity = $\frac{\text{True positive}}{\text{False Positive + True negative}} \times 100$

4. Negative Predictive value = $\frac{\text{True negative}}{\text{False negative + True negative}} \times 100$

CONCLUSION

The percent accuracies, false positive and false negative results of indirect tests *viz* CMT, SCC, ER and pH to detect subclinical mastitis in 629 milk samples were studied taking Cultural test as standard. The tests are fairly in good agreement with bacterial culture. But estimation of SCC was more accurate test after cultural isolation, followed by CMT.

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