RESEARCH ARTICLE
Open Access

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2022.53.4.

Comparison of California Mastitis Test and somatic cell counts for detection of subclinical mastitis in crossbred cattle

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Citation: Arifa, A., Meganath, K.V., Vignesh, S., Rojan, P.M., Muhammed, E.M., Rathish, R.L., Muhasim Asaf, V. N. and Dinesh, C.N. 2022 Comparison of California Mastitis Test and somatic cell counts for detection of subclinical mastitis in crossbred cattle. *J. Vet. Anim. Sci.* **53**(4):
DOI: https://doi.org/10.51966/jvas.2022.53.4.

Received: 29.06.2022 *Accepted: 03.09.2022* *Published: 31.12.2022*

Abstract

Mastitis is a major constraint that severely affects milk production in dairy animals. The California Mastitis Test (CMT) is a reliable and rapid field test for the diagnosis of subclinical mastitis (SCM) which gives an indirect estimate of somatic cell count (SCC). Based on the results of CMT screening and SCC of the milk of 105 crossbred animals located in different farms in Wayanad and Calicut districts of Kerala state, the present study attempts to find the estimates of sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV), false discovery rate (FDR) and false omission rate (FOR) of CMT relative to SCC as the reference standard. The correlation and agreement between CMT and SCC were also analysed. The estimated Spearman and Kendall Tau b correlation coefficients were 0.88 and 0.76, respectively, which indicated a strong positive relationship between CMT and SCC. The sensitivity and specificity values of CMT were 1.000±0.000 and 0.510±0.071, respectively. These values indicate that the probability for an animal with mastitis to be identified using CMT is 100 per cent and the probability of correctly identifying an animal without mastitis animal is 51 per cent. The high sensitivity value of CMT in this study indicated that CMT could be used to find out the true prevalence of SCM in crossbred animals. Analysis of the data also revealed that CMT had a PPV of 0.700±0.051 and an NPV of 1.000±0.000. The calculated accuracy of CMT was 0.771±0.041. The estimated FDR and FOR were 0.300±0.051 and 0.000±0.000, respectively. Kappa statistic was used to determine the level of agreement between CMT and SCC and the kappa coefficient value was 0.53±0.07 which indicated moderate agreement.

Keywords: *Subclinical mastitis, California Mastitis Test, somatic cell count, correlation*

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Mastitis is a major disease in dairy cattle affecting milk production and the health of the animal. It is prevalent worldwide and responsible for nearly 70 per cent of loss in milk production (https://www.financialexpress.com / market/commodities/bovine-mastitis-resulting-in-70-per-cent-loss-in-milk-production-IIT-Roorkee-study/2062540/ Accessed on 11 February 2022). In India, the annual loss due to mastitis was estimated to be around U.S. $ 971.39 million (Audarya, *et al*., 2021).

Depending on the visibility of the effects of inflammation of the mammary gland, mastitis can be classified as clinical and subclinical. In clinical mastitis (CM), symptoms like swelling, heat, hardness, redness, and pain on the udder as well as change in the colour and consistency of milk would be obvious. However, subclinical mastitis (SCM) does not produce any visible effects on the udder or milk quality. But it has important effects on milk composition, manifested mainly as an increase in the somatic cell count (SCC) (Karimuribo *et al*., 2006; Iraguha *et al.*, 2015). Even though symptoms are less drastic in SCM, its impact on milk production is equally the same as that of CM. Moreover, in dairy animals, SCM is more prevalent than CM. In dairy cattle and buffaloes, the pooled prevalence of SCM and CM globally were 42 per cent and 15 per cent, respectively. The corresponding prevalence of SCM and CM in India were 45 per cent and 18 per cent, respectively (Krishnamoorthy *et al*., 2021).

For treatment and control, the mastitis condition needs to be diagnosed without any delay. The California Mastitis Test (CMT) is an efficient, reliable and rapid field test for the diagnosis of SCM on the cow side (El-Balkemy *et al*., 1997). This simple test was developed by Schalm and Noorlander (1957), and gives an indirect estimate of SCC because it is based on a gelling reaction between the nucleic acid of the cells and a detergent reagent.

Somatic cells are predominantly milk-secreting epithelial cells that have been shed from the lining of the gland and white blood cells that have entered the mammary gland in response to injury or infection (Sharma *et al*., 2012). Currently, the SCC is accepted as the international standard for the measurement of milk quality. According to the National Mastitis Council Guidelines (2001), normal milk from uninfected or uninflamed mammary quarters would have an SCC of less than 100,000 cells/mL. When the SCC is equal to or exceeds 200,000 cells/mL and bacteria are isolated in the absence of clinical changes, then that udder quarter is defined as being sub-clinically infected. Clinically infected mammary quarters would almost always have SCC greater than 200,000 cells/mL. According to Sharma *et. al*. (2012), the health status of the udder could be graded into healthy (0 to 2,00,000 cells/mL), sub-clinical mastitis (2,00,000 to 1,200,000 cells/mL) and clinical mastitis (> 1,200,000 cells/mL) based on the SCC. Thus, SCC is the most widely used single reliable indicator of udder health and is a useful predictor of intramammary infection. Since, the SCC in milk is readily available, the pricing of milk in several European countries is based on its SCC.

The present study attempts to find the estimates of sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV), false-positive probability (or rate) (FPR) and false-negative probability (FNP) of CMT relative to SCC as the reference standard. The correlation and agreement between CMT and SCC were also analysed. The FPR is also referred to as False Discovery Rate (FDR) and the FNP is also called the False Omission Rate (FOR).

Materials and methods

The animal population under the study included a total of 105 lactating crossbred cows maintained in the cattle farm of the Instructional Livestock Farm Complex of Kerala Veterinary and Animal Sciences University, Pookode and two private farms located in Wayanad and Calicut districts.

Approximately 3 to 5 mL of milk was stripped from individual udder quarters into the shallow cups of the CMT paddle for CMT. An equal amount of CMT reagent was added to the shallow cups, mixed by gentle rotation in a horizontal plane for about 10-30 sec. The CMT score and the interpretation of the reaction results suggested by Sharma *et al.* (2012) are modified and grades are assigned to facilitate a comparison of CMT with SCC (Table 1).

Then another 5mL milk sample was collected from individual quarters of each animal in 15mL centrifuge tubes and the samples were transported to the laboratory within one hour at room temperature. The SCC was found using the DeLaval cell counter DCC.

Statistical analysis

The correlation between CMT grades and SCC in the milk was calculated using the CORR procedure in SAS® OnDemand for Academics (SAS Institute Inc. 2014). For analysis of other estimates, the CMT grade 1 was considered as healthy (or negative) and grades 2,3,4, and 5 were grouped as infected (or positive) as shown in Table 1. Moreover, for analysing the results of the present study, the animals were classified as healthy (or negative) with SCC scores below 2,00,000 cells/mL and infected (or positive) with SCC scores above 2,00,000 cells/mL.

The sensitivity, specificity, accuracy, PPV, NPV, FDR and FOR of CMT were calculated using the SAS PROC FREQ procedure after the categorisation of CMT and SCC results as mentioned above. For the calculation of these estimates, SCC was considered the reference standard. Moreover, Kappa statistics as a test for the agreement was also estimated.

Results and discussion

The results of the CMT screening and SCC of milk samples are given in Table 2 and Table 3. None of the animals under study showed clinical symptoms of mastitis.

The correlation between CMT grades and SCC were found to be 0.88 (Spearman correlation coefficient) and 0.76 (Kendall Tau b correlation coefficient). The correlation analysis revealed a strong positive relationship between CMT grades and SCC readings. Both these coefficients were statistically significant at p <0.0001. However, a study by Salvador *et al*. (2013) on the milk of water buffaloes in the Philippines showed a weak positive correlation between these two tests. Further, Jorge *et al*. (2005), reported a 53 per cent correlation between the two tests. However, a higher correlation between the CMT and SCC tests was also reported in several studies conducted on the milk of dairy cattle, goats and camels (Perrin *et al.*, 1997; Costa *et al*., 2000; Abdul-Gadir *et al., 2006;* Hamman *et al*.,2010).

Table 2. Classification of animals based on CMT

CMT Score	Assigned Grades	Number of animals
Negative	1	25 (23.81)
Trace	2	25 (23.81)
Weak	3	20 (19.05)
Distinct	4	34 (32.38)
Strong positive	5	1 (0.95)

Values in the parenthesis are the percentage

Table 3. Classification of animals based on SCC

SCC	Number of animals
0 to 2,00,000 cells/mL	49 (46.67)
2,00,000 to 1,200,000 cells/mL	40 (38.10)
> 1,200,000 cells/mL	16 (15.24)

Values in the parenthesis are the percentage

Table 1. The CMT scores and interpretation of the reactions

CMT score	Assigned grades	Description	Interpretation
Negative	1	No change	Healthy quarter
Trace	2	Slime formed which disappeared with continuous movement of the paddle.	Infected quarter
Weak	3	Distinct slime, but no gel formation	
Distinct	4	Viscous with gel formation, which is adhered to the margin	
Strong positive	5	Gel formation with convex projection. The gel did not dislodge after the swirling movement of the paddle.	

Table 4. Results from CMT and SCC

	SCC Results		
CMT Results	Positive (Infected)	Negative (Healthy)	Total
Positive (Infected)	56	24	80
Negative (Healthy)	0	25	25
Total	56	49	105

Table 5. Estimates of CMT considering SCC as the reference standard

	Estimate ± SE
Sensitivity	1.000±0.000
Specificity	0.510±0.071
Accuracy	0.771±0.041
PPV	0.700±0.051
NPV	1.000±0.000
FDR	0.300±0.051
FOR	0.000±0.000

For determining the sensitivity, specificity, accuracy, PPV, NPV, FDR and FOR the results of CMT and SCC were compiled as given in Table 4 and the estimated values are given in Table 5.

The sensitivity is the proportion of true positive responders that have a positive test result. The specificity is the proportion of true negative responders that have a negative test result. The sensitivity and specificity values for CMT were 1.000±0.000 and 0.510±0.071, respectively. These values indicate that the probability for a mastitis animal to be identified using CMT is 100% and the probability of correctly identifying a non-mastitis animal is 51 per cent.

Based on the study on the milk of buffaloes, Salvador *et al.* (2013) reported a sensitivity of 54.43 per cent and a specificity of 77.10 per cent for CMT. However, the sensitivity for CMT found in this study is high which is comparable to some earlier studies. Sensitivity values of 96 per cent, 86.07 per cent and 82.4 per cent were reported by Tanwar *et al*. (2001), Sharma *et al*. (2010) and Dingwell *et al*. (2003), respectively. However, bacterial culture was used as the basis in the studies of Dingwell *et al*. (2003) and Sharma *et al*. (2010) whereas SCC was used as the standard in the present study. A high sensitivity value for CMT in this study indicates that CMT can be used to find out the true prevalence of subclinical mastitis in crossbred animals.

However, the specificity value for CMT in the present study was low when compared to some earlier reports. The specificity values of 59.70%, 77.10%, 80.6% and 90.4% for CMT were reported by Sharma *et al.* (2010), Salvador *et al*. (2013), Dingwell *et al*. (2003) and Ozenc *et al*. (2008), respectively.

The PPV is the proportion of positive test results that are true positive responders. It is the probability that a disease is present in animals with positive test results. The NPV is the proportion of negative test results that are true negative responders. It is the probability that disease is absent in animals with negative test results (Salvador *et al*., 2013). The analysis of the data revealed that CMT had a PPV of 0.700±0.051 and an NPV of 1.000±0.000. The calculated accuracy of CMT was 0.771±0.041. These values were high compared to the PPV, NPV and calculated accuracy of CMT reported by Salvador *et al*. (2013), who found the values as 42.11%, 84.67% and 71.78%, respectively.

The FDR is the proportion of true negative responders that have a positive test result. The FOR is the proportion of true positive responders that have a negative test result. In the present study, the estimated FDR and FOR were found to be 0.300±0.051 and 0.000±0.000, respectively.

Kappa statistic is used to determine the level of agreement between CMT and SCC (Salvador *et al*., 2013). The simple kappa coefficient (Cohen, 1960) is a measure of agreement. The Kappa value interpretation according to Landis and Koch (1977) were: <0 - No agreement; 0.0 to 0.20- Slight agreement, 0.21 to 0.40- Fair agreement; 0.41 to 0.60- Moderate agreement; 0.61 to 0.80- Substantial agreement; 0.81 – 1.0- Perfect agreement. In the present study, the kappa coefficient value

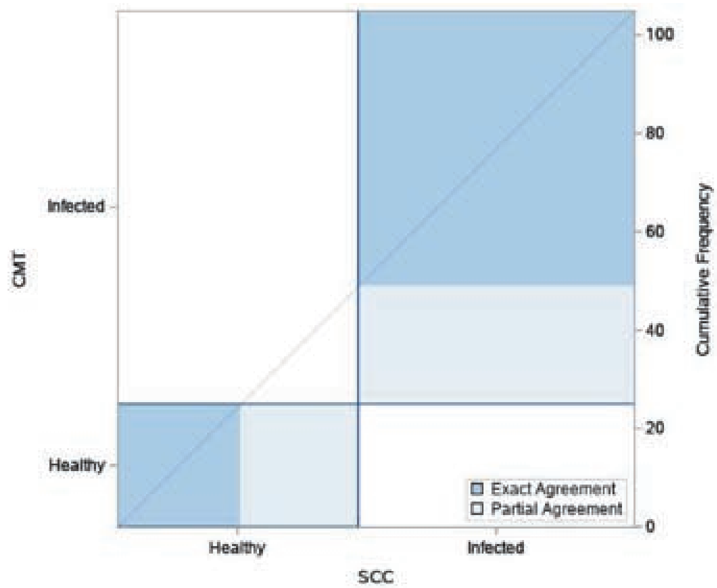

Fig. 1. Agreement plot of CMT and SCC

was found to be 0.53±0.07 which indicates moderate agreement. The agreement plot between CMT and SCC is depicted in Fig.1. In a study conducted by Salvador *et al*. (2013) on Murrah buffalo milk, they found the level of agreement between the CMT and SCC results as slight.

The strength of association between CMT and SCC from the contingency table was measured using phi statistic, and the value obtained was 0.598, indicating a strong positive relationship.

Conclusion

From the study, it was found that the sensitivity value for CMT was 1.000±0.000 which indicates that the probability for a mastitis animal to be identified using CMT is very high. This means that CMT can be used as an efficient, reliable and rapid field test for the diagnosis of SCM on the cow- side in tropical field conditions.

Acknowledgement

We are thankful for the Kerala Veterinary and Animal Science University for the support and facilities to carry out this study.

Conflict of Interest:

The authors declare that they have no conflict of interest.

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