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Comparative Accuracy of Cow-Side Tests for Detection of Subclinical Mastitis

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ABSTRACT

Subclinical mastitis (SCM) greatly influences the udder health, lowers milk quality and quantity. The gross clinical signs being absent, it is left undetected leading to high economic impacts on dairy farming. Cow-side indirect tests are employed for diagnosis besides the bacteriological culture (BC) as the gold standard. This study aimed to evaluate the diagnostic validity/ accuracy of cow-side tests like California Mastitis Test (CMT), Somatic Cell Count (SCC) and Electrical Conductivity (EC) with BC as the gold standard test for SCM detection. Growth on culture media of at least one colony of a major pathogen was taken as the criterion for a positive test. A total of 410 quarter milk samples were tested. The accuracy and Cohen's kappa values for CMT were 70.98% and 0.42, for SCC 76.83% and 0.56, and for EC 53.45% and 0.09. The Receiver Operating Characteristic analyses showed the area under curve for CMT, SCC and EC as 78.10%, 81.20% and 59.90%, respectively. Both CMT and SCC had a good agreement with the gold standard test with SCC showing superiority over CMT in terms of diagnostic validity/ accuracy. Thus, the study endorsed SCC as the first choice for accurate detection of SCM in dairy cattle followed by CMT.

HIGHLIGHTS

- CMT, SCC and EC were compared for subclinical mastitis detection.
- SCC was superior to CMT with more kappa value and area under curve.

Keywords: Subclinical mastitis, Bacteriological culture, Cow-side Indirect Tests, Diagnostic Accuracy

Mastitis, a prevalent and economically significant disease in dairy cattle, is characterized by inflammation of the mammary gland, resulting in physical, chemical, and pathological changes in milk and udder tissues (Constable *et al.,* 2017). This inflammatory response, marked by increased blood proteins and white blood cells in mammary tissue, aims to eliminate irritants, repair damage, and restore udder function. However, it leads to reduced milk yield and quality, premature culling, and elevated

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veterinary costs, posing a substantial global challenge to dairy farming (Nielsen *et al.,* 2010; Hogeveen *et al.,* 2011; Ruegg, 2017; Aghamohammadi *et al.,* 2018).

Mastitis in dairy cattle predominantly occurs in subclinical form, characterized by increased milk leukocyte and bacterial counts and reduced milk production, without visible signs of inflammation or abnormalities in milk (Halasa *et al.,* 2007). Therefore, SCM is left undiagnosed and untreated causing the infection to spread other quarters and cows. Majority of the intramammary infections (IMI) in dairy cattle are in subclinical form rather than the clinical form (Reyher *et al.,* 2011; Goncalves *et al.,* 2018) and this subclinical form being the most prevalent worldwide with the highest economic burden (Petrovski *et al.,* 2006; Bradley *et al.,* 2007). Clinical mastitis can be diagnosed by examining the udder and milk for visible abnormalities (Oliver *et al.,* 2004). However, indirect tests are required for subclinical mastitis diagnosis which detect the increase in the concentration of immune cells and immune mediators during the inflammatory process (Jaeger *et al.,* 2016; Hughes and Watson, 2018). There are other inflammatory markers like some specific proteins which have been less explored for their diagnostic efficiency for SCM but they do not fit to the cow-side test category. For a profitable dairy farming and optimum health of dairy cattle, accurate detection of SCM is imperative.

SCM is routinely diagnosed by cow-side tests California mastitis test (CMT), somatic cell count (SCC) and electrical conductivity (EC). CMT, a qualitative measurement of the somatic cell count in milk, is a screening test that is simple, inexpensive, and can be used easily at cow-side for mastitis detection (Leslie *et al.,* 2002; Dingwell *et al.,* 2003). SCC represents the immune cells in milk like neutrophils, macrophages, lymphocytes and some epithelial cells (Damm *et al.,* 2017) and is an important and practical tool for assessing udder health of a herd or individuals. EC of milk detects mastitis since the ionic changes like increase in sodium and chloride content of milk occur during inflammation (Korhonen *et al.,* 1995). Therefore, these indirect tests need to have comparison for their Accuracy with the gold standard test like bacteriological culture (BC), for early disease diagnosis and prompt intervention (Crosson *et al.,* 2015).

This study was undertaken to ascertain the diagnostic accuracy of CMT, SCC and EC for SCM detection with bacteriological culture as the reference test for SCM.

MATERIALS AND METHODS

Sample collection

For comparative study, 410 quarter milk samples were collected from 52 cows (cross bred jersey) at Mountain Livestock Research Institute, Mansbal SKUAST-Kashmir (MLRI) and from 78 cows at the Veterinary Clinical Complex (VCC), Faculty of Veterinary Sciences Shuhama. Only those cows were selected for sampling which were suspected to suffer from SCM based on the case definition framed for SCM. About 10 mL milk was collected aseptically after disinfection of teat surface with 70% ethanol soaked cotton in separate sterile wide mouth vials after discarding 2-3 streams. The samples were stored in an ice box and immediately transported to the Division of Veterinary Epidemiology and Preventive Medicine laboratory for testing.

Bacteriological culture of milk

Bacteriological cultures were carried out according to the standard microbiological procedures of inoculation and incubation (International Dairy Federation, 1981; Jaeger *et al.,* 2016). 10 µL of milk were cultured on nutrient agar, blood agar and MacConkey agar. Plates were incubated at 37°C and examined 24-48 h later. Colonies were identified by morphology and Gram staining. Only major pathogens of mastitis were considered for positive test. The sample was considered positive for SCM if at least one colony (1 cfu/ml) of a major pathogen was detected (Gerardi *et al.,* 2009).

California Mastitis Test

CMT was conducted on fresh and unrefrigerated milk immediately after collection as per the standard procedure (Schalm and Noorlander, 1957; Ikram, 1997). 5ml milk was dispensed in CMT paddle and an equal volume of CMT reagent was added to all the 4 parts of the paddle containing milk. The milk was visualized for any change in the colour and consistency in terms of gel formation and its viscosity. The grading of the reaction was done by the intensity of gel formation.

Electrical Conductivity

EC was measured on fresh and unrefrigerated milk

immediately after collection by hand-held Draminski Mastitis Detecter, Poland (Iraguha *et al.,* 2017; Amritha Priya, 2021). 5 mL milk was taken in the instrument cup and results interpreted as per the manufacturer's instructions.

Somatic Cell Counts

SCC was done by Somatic Cell Counter (DeLaval, Sweden) as per the manufacturer's instructions. SCC greater than threshold level of 200,000 cells/mL was taken as the cut-off to differentiate healthy and subclinically affected quarters (National Mastitis Council, USA, 2001; Salvador *et al.,* 2014).

STATISTICAL ANALYSIS

Confusion matrix tables were drawn for comparing CMT, SCC and EC with BC for calculation the validity/accuracy criteria. Cohen's kappa values were calculated for estimating the 'levels of agreement' between the efficacies of the tests.

RESULTS AND DISCUSSION

Out of 410 quarter milk samples collected, 174 samples were positive for major mastitis pathogens as identified by morphological and staining characteristics of the pathogen (Table 1). These samples were regarded as positive for SCM.

Table 1: Bacteriological culture results

Parameter	MLRI	VCC
Quarter milk samples	166	244
No of Cows Screened	52	78
BC positive samples	63 (37.95%)	111 (45.50%)
S aureus	29 (46.03%)	47 (42.34%
Streptococci	15 (23.80%)	21 (18.91%)
E. coli	6 (9.50%)	14 (12.61%)
Mixed Growth	13 (20.63%)	29 (26.12%)

Somatic Cell Count

SCC of >2 × 10^5 cells/mL was taken as the cut-off to assign the sample as positive. The mean SCC (× 10^5 cells/mL) of positive samples was 3.18±0.27 and that of negative samples was 1.06±0.08. The difference was significant (P<0.05). Out of 410 samples, 146 samples were positive for SCC (Fig. 1). The Se, specificity Sp, PPV, NPV and accuracy were 83.90%, 73.30%, 69.85%, 86.07% and 77.80%, respectively. Cohen's kappa and McNemar's test values were 0.56 and 0.003 (Table 2). The ROC curve shows the AUC as 0.812 (Fig. 4).

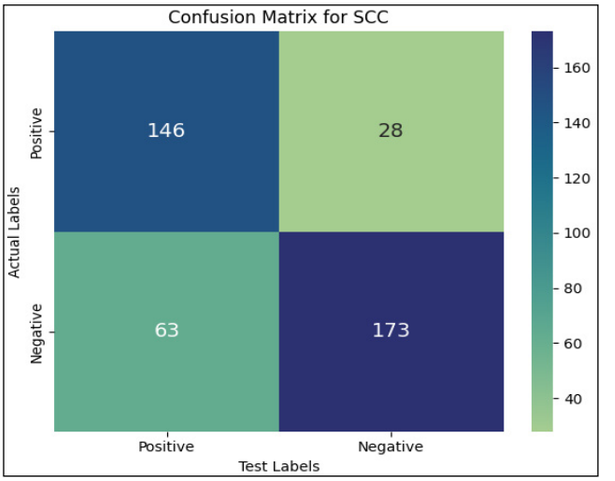

Fig. 1: Confusion matrix for SCC

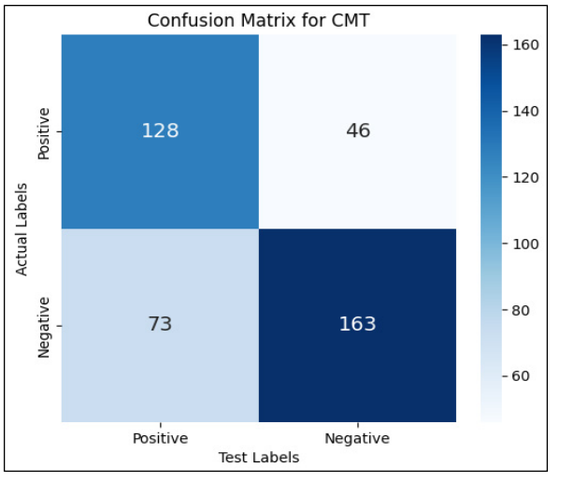

Fig. 2: Confusion matrix for CMT

California Mastitis Test

CMT was visualized for any change in the colour and consistency in terms of gel formation and its viscosity.

CMT score of 1 or more was considered as positive. Out of 410 samples, 128 samples were positive on CMT (Fig. 2). The Sensitivity (Se), Specificity (Sp), Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Accuracy of the tests was 73.56%, 69.07%, 63.68%, 78.00% and 70.97%, respectively. Cohen's kappa and McNemar's test values were 0.42 and 0.016 (Table 2). The Receiver Operating Characteristic (ROC) curve shows the area under curve (AUC) as 0.781 (Fig. 4).

Electrical Conductivity

On EC, out of 410 samples, 98 samples were positive for SCM (Fig. 3). The Se, Sp, PPV, NPV and Accuracy of the tests were 56.32%, 53.39%, 47.11%, 62.37% and 54.63%, respectively. Cohen's kappa and McNemar's test values stood at 0.09 and 0.015 (Table 2). The ROC curve shows the AUC as 0.599 (Fig. 4).

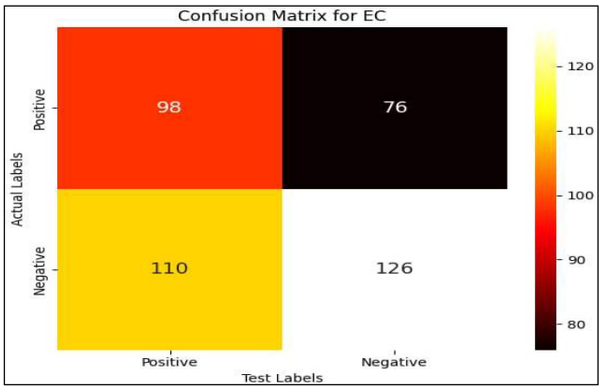

Fig. 3: Confusion matrix for EC

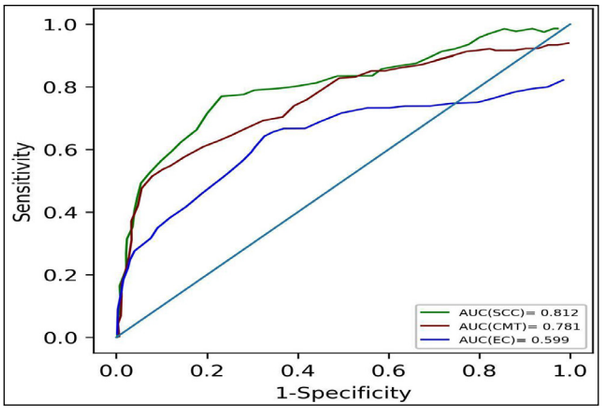

Fig. 4: Receiver Operating Characteristic curve of CMT, SCC and EC

Mastitis is an inflammatory disease of udder with multifactorial etiology and is the most frequent disease of dairy cattle with high economic importance leading to huge losses worldwide including India (Hogeveen *et al.,* 2011; Bardhan, 2013). Pathogenic microbes invade the mammary tissue causing inflammation in the form of clinical mastitis with overt signs of udder and milk abnormalities or subclinical mastitis with no visible or gross changes of udder or milk (Talbot and Lacasse, 2005). SCM is of immense importance as the systemic and local signs of inflammation are absent and the milk is apparently normal but with decreased production and compromised quality (Salvador *et al.,* 2014). For optimum health of dairy cattle and a profitable and sustainable dairy farming, early detection of SCM through highly-sensitive/specific and affordable cow-side diagnostic tests is important.

A comparative study was carried out to evaluate various diagnostic tests like CMT, SCC and EC with cultural examination/bacteriological culture (BC) of milk for their diagnostic accuracy. BC is costly, time consuming with a limited applicability under field conditions and is not feasible as a field test (cow-side) test (Iraguha *et al.,* 2017). BC of milk was considered the 'gold standard' test for SCM to which other tests were compared (Dohoo *et al.,* 2011). It is the standard method for identifying IMIs (National Mastitis Council, 1999). Of 410 milk samples tested, 174 (42.44%) were subclinically effected. In this study, the milk sample was considered BC positive if at least one colony of any major pathogen was detected. A similar criterion was followed by Nyman *et al.,* 2014 for categorizing a milk sample as culture positive or negative. A sample is regarded as BC positive/SCM if at least one colony is visible on milk culture (Gerardi *et al.,* 2009; Cunha *et al.,* 2020). Jashari *et al.* (2015) found that only ≥1 colony (100 cfu/mL) of a major pathogen on milk culture had a high correlation with SCC for intramammary infection causing SCM. Jaeger *et al.* (2016) also determined the culture positivity of milk samples by detection of at least on colony of a major pathogen. A quarter is subclinically infected if there is growth of any major pathogen on milk bacteriology culture (Dohoo *et al.,* 2011). Similar recommended criteria have been followed in this study to determine a sample as culture positive.

SCC greater than threshold level of 2 x 10^5 cells/mL was taken as the cut-off to differentiate healthy and subclinically affected quarters (National Mastitis Council, USA, 2001;

Table 2: Diagnostic Accuracy of CMT, SCC and EC

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	*Cohen's*	McNemar
CMT	73.56	69.06	63.67	78.00	70.98	0.42	0.016
SCC	78.16	75.85	70.46	82.49	76.83	0.56	0.003
EC	56.32	51.33	46.03	61.46	53.45	0.09	0.015

Petzer *et al.,* 2017). This threshold was chosen to lessen the false negative results and achieve an acceptable sensitivity and specificity and to lessen the false-negative results. This cut-off corresponds with the study of Jashari *et al.* (2015) who achieved comparable specificity with bacteriological culture taken as the standard. A cut-off SCC of >2 x 10^5 cells/mL is the standard for separating healthy from mastitis affected milk and to reduce the diagnostic error (Madouasse *et al.,* 2010; Petzer *et al.,* 2017). SCC value of <2 x 10^5 cells/mL is taken as a threshold for uninfected milk samples (Schukken *et al.,* 2003). SCM is very often diagnosed based on SCC threshold level (Sumon *et al.,* 2020) and is good indicator of IMI (Akerstedt *et al.,* 2007; Alhussein and Dang, 2018). In this study, the Se, Sp, PPV and NPV values for SCC were 83.90%, 73.30%, 69.85% and 86.07%, respectively, with an accuracy of 77.80%. Cohen's *Kappa* and McNemar values were 0.56 and 0.003, which were in agreement with the BC. From ROC analysis, the AUC was 0.812. Reddy *et al.* (2014) found the lowest Se (56.62%) for EC but the Sp was highest (84.84%) when compared with CMT and SCC. Se (39.80%) and Sp (84.80%) was observed for SCC by Langer *et al.* (2014). Dasohari *et al.* (2018) observed Se (69.49%), Sp (78.57%) and PPV (77.36%) for SCC based diagnosis of SCM in dairy cows. These variations for comparative results can be attributed to the reason that the cut-off SCC value was taken as 3,00,000 cells/mL in contrast to this study (2,00,000 cells/mL) which could show false negative results in SCC determination. Further, SCC in milk is affected by the type of bacteria present which lead to variations in SCC in individual animals also (Sumon *et al.,* 2020).

In this study, most of the BC positive samples showed at least 1+ reaction on CMT although the true negative detection was less. CMT revealed a Se and Sp of 73.56% and 69.06%, respectively. The PPV and NPV was 63.68% and 78.00 %, respectively with an accuracy of 70.97%. Cohen's *Kappa* and McNemar values were 0.42 and 0.016 which showed the level of agreement to the gold standard test. From ROC analysis, the AUC was 0.781. Iraguha *et al.* (2017) found CMT the preferred cow-side test with a Se (88.46%) and Sp (86.17%) and kappa agreement 'substantial' (k = 0.66) after SCC for SCM detection. CMT has been found to have a Se and Sp of 82.40% and 80.60%, respectively for IMI detection in comparison to BC of major pathogens as the gold standard test (Dingwell *et al.,* 2003). Langer *et al.* (2014) observed a Se and Sp of 60.10% and 62.70%, respectively for CMT for SCM diagnosis in dairy cows. Dasohari *et al.* (2018) reported Se of 74.58%, Sp of 78.57% and PPV of 78.57 for SCM by CMT. CMT is a handy and valuable test for subclinical mastitis and is considered a characteristic indicator of mammary gland infection (Souza *et al.,* 2012).

In the present study, EC revealed Se, Sp, PPV, NPV and Accuracy of 56.32%, 53.39%, 47.11% and 62.37% and 54.63%, respectively. *Kappa* and McNemar values were 0.09 and 0.015. From ROC analysis, the AUC was 0.599. These lower values compared to other tests can be due to the reason that EC is based on the detection of chemical changes like increased influx of sodium and chloride ions into the lumen due to increased permeability of capillaries which is more pronounced in clinical mastitis. Langer *et al.* (2014) also reported that EC has the least efficacy for detection of SCM. Ruegg *et al.* (2002) on comparison of cow-side tests found CMT superior over EC for subclinical mastitis detection and that the predictive value of EC is poor. The variations in EC values are also affected by other diseases and milk fat (Amritha Priya *et al.,* 2021). EC also has the limitation that its values in milk are prone to non-mastitic problems also (Hamann and Zecconi, 1998). EC has not been found reliable for SCM detection (Nielen *et al.,* 1995). Similar results of least Se and Sp with EC test of milk have been observed in the present study.

CONCLUSION

This study highlights SCC as the most reliable and accurate test for subclinical mastitis detection, demonstrating

the closest agreement with bacterial culture results, as supported by its superior ROC-AUC and higher kappa value. SCC outperformed other diagnostic methods, including the California Mastitis Test and electrical conductivity, which showed the least agreement with bacteriological culture. Therefore, SCC should be prioritized for routine monitoring to effectively identify subclinical infections and maintain udder health in dairy herds.

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