Journal of Applied Veterinary Sciences, 10 (2): 76-85 (April, 2025).
ISSN: Online: 2090-3308, Print: 1687-4072
Journal homepage : https://javs.journals.ekb.eg

Comparative Evaluation of Some Diagnostic Tests for on-farm Screening of Subclinical Mastitis in Crossbreed Dairy Cows

Sangwa A. L[1, 2*], I.P. Kashoma[3] and A.M. Lupindu[1]

[1]Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, P. O. Box 3021, Morogoro, Tanzania

[2]Mbarali District Council, P.O. Box 237, Rujewa, Tanzania

[3]Department of Veterinary Surgery and Theriogenology, Sokoine University of Agriculture, P.O. Box 3020, Morogoro, Tanzania

***Corresponding Author:** Anania Lawrance Sangwa, **E-Mail:** ananiasangwa@gmail.com

ABSTRACT

Subclinical mastitis is a costly disease for dairy cattle worldwide due to economic losses from reduced milk yield, veterinary costs, and premature culling; thus, regular screening is of paramount importance for early detection, prompt treatment, and effective control measures. This study was conducted to assess the possible risk factors and compare the efficiency of three indirect mastitis diagnostic tests for their ability to classify correctly under health status in individual cows. Seven hundred and forty-two milk samples from 186 lactating cows kept in six farms were screened for subclinical mastitis using Sodium Lauryl Sulfate (SLS), California Mastitis Test (CMT), and Somatic Cell Count (SCC). The research revealed moderate overall prevalence (21.5%) of farm-level subclinical mastitis with high (65.2%) prevalence in farm B and low (10.7%) in farm F. Specific test-based results were 36.56%, 36.56% and 21.51% for SLT, CMT and SCC, respectively. There was a statistical difference (p=0.0001) between SCC and SLS and between SCC and CMT, but no significant differences (p=0.57) were observed between SLS and CMT. Quarter subclinical mastitis prevalence for SLS, CMT, and SCC was 16.85% (95% CI: 14.3-19.7), 15.77% (95% CI: 13.1-18.3), and 8.45% (95% CI: 6.4-10.5), respectively. Both SLS and CMT demonstrated strong sensitivity (100% and 90.48%) and specificity (90.72% and 91.16%) compared to the SCC test. Positive predictive values for SLS and CMT were 50% and 48.72%, respectively, whereas negative predictive values for SLS and CMT were 100% and 99.04%. Cohen's Kappa of SLS was 0.62, while CMT showed 0.58. The area under the receiver operating characteristic curve for SLS and CMT was 0.996 and 0.997, respectively. The diagnostic accuracy of SLS and CMT were 91.51 and 91.11, respectively. Based on the diagnostic efficiency of SLS in terms of sensitivity, specificity, predictive values, and kappa index, it is suggested that SLS can be used as an alternative to CMT for animal-side subclinical mastitis diagnostic tests.

Original Article:
DOI:https://dx.doi.org/10.21608/javs.2025.351733.1517

Received : 17 January, 2025.
Accepted: 06 March, 2025.
Published in April, 2025.

Keywords: Anionic surfactant, Dairy cows, Sodium, Aryl Lauryl Sulfonate Subclinical mastitis.

J. Appl. Vet. Sci., 10(2): 75-85.

INTRODUCTION

Mastitis can be classified into clinical and subclinical forms based on the visibility of the effects of inflammation **(Cobirka *et al.*, 2020)**, and acute, sub-acute, and chronic depending on its severity **(Baeza, 2016)**. Clinical mastitis involves evident abnormalities in milk, physical changes in the udder, and systemic symptoms in the cow. Meanwhile, subclinical mastitis doesn't manifest visible effects on the udder or milk quality but still influences milk composition, notably increasing Somatic Cell Counts (SCC) **(Tommasoni *et al.*, 2023)**. Bovine subclinical mastitis is a prevalent and economically significant disease affecting dairy cattle worldwide. On a global scale, subclinical mastitis is a major contributor to the economic losses associated with mastitis and decreasing milk yield in dairy cattle breeding **(Çýlek and Tekýn, 2007; Çilek and Gotoh, 2012)**. The losses are substantial exceeding £1/2 billion (538 701 559.59 US Dollars) annually **(Andrews, 2004). Komba and Kashoma, (2020)** conducted a study at Kitulo Livestock Multiplication Unit, Magadu and Mazimbu farms; they found that the estimated financial losses per farm resulting from the presence of subclinical mastitis ranged from 1,326,000 to 7 446 000 Tanzanian shillings (577.00–3 239.00 US Dollars) annually with an average quarter loss attributed to subclinical mastitis being 49 320 Tanzanian shillings (21.45 US Dollars). Therefore, early detection of SCM is the key component of the mastitis control program **(Kour *et al.*, 2023).**

Dairy cattle mastitis is a management-related disease with its prevention and control sorely depending on many factors including the type of management practiced on the farm **(Kivaria, 2006).** Thus, early detection of the disease is the key component of a mastitis control program. Currently, various diagnostic tests for detection of subclinical mastitis have been developed such as California Mastitis Test (CMT), Modified White Side Test (WST), Surf Field Mastitis Test (SFMT), electrical conductivity of milk, Cl- estimation in milk, Modified Aulendorfer Mastitis Probe (MAMP) test, Somatic cell count (SCC), n-Acetyl-B-DGlucose minidase (NAGase) enzyme activity, ELISA, and bacterial culture **(Tanni *et al*., 2021b; Tommasoni *et al*., 2023).** Although bacteriological culture of milk samples is considered the gold standard method to identify intramammary infection, it does not measure the degree of inflammation associated with the infection **(Dohoo *et al.*, 2011).** Since subclinical mastitis (SCM) is not only associated with infections, the inflammation of the mammary gland can be detected directly by the increase in somatic cell count **(Alhussien and Dang, 2018).**

Furthermore, of the indirect cow-side screening tests, CMT has been considered as most sensitive and specific screening test for subclinical mastitis **(Godden *et al.*, 2017; Tanni *et al*., 2021b)**. The reagent (Sodium alkyl aryl sulfonate) used in the CMT is an anionic surfactant that works by decreasing the milk surface tension, changing cell membrane and nucleus conductivity, interfering with osmosis and finally increasing milk viscosity **(Mesa and Risuleo, 2021).**

Alternatively, Sodium Lauryl Sulfate (SLS) is another potential anionic surfactant with similar functions to Sodium Alkyl Aryl Sulfonate **(Tanni *et al.*, 2021a)** which is easily available and cheap; used as a test reagent to screen subclinical mastitis in dairy cows in the Philippines **(Waminal, 2021).** SLS has a similar action with Sodium Alkyl Aryl Sulfonate **(Tanni *et al*., 2021a)** and when NaOH is added a faster degradation of somatic cells occurs exposing DNA of the cells leading to formation of gel in mastitic milk **(Sharif and Muhammad, 2009).** However, in developing countries such as Tanzania, commercial CMT reagents are not readily available whenever needed and the cost of imported CMT kits limits the cost-benefit for diagnosis and treating subclinical mastitis under the smallholder farmers' setup. Thus, the current work aimed to assess the efficiency of Sodium Lauryl Sulfate surfactant as an alternative screening test for subclinical mastitis in comparison with other cow-side screening tests in dairy cows maintained under farmer's management around Morogoro Municipality.

MATERIALS AND METHODS

Ethical approval

Ethical approval for this study was granted by the Tanzania Livestock Research Institute (TLRI), under reference number TLRI/CC.21/039.

Study area

This study was conducted in Morogoro Municipality, Tanzania. Geographically, Morogoro municipality spreads between longitude 35.6 to 39.5° E and latitude 5.7 to 10° S and is elevated between 500 to 600 m above sea level. Climatically, the area has ambient temperature ranges from 27 to 31°C with a minimum night temperature of 14°C in the coolest months. The municipality experiences a sub-humid tropical climate with a bimodal rainfall pattern characterized by two rain seasons in a year with a dry season separating the short rains (October to December) and long rains (which fall from March to May/June). The average annual range is between 500 and 1800 mm, with about 83% of the rain falling between late February and the end of May.

Study Animals and their Management

A total of six medium-scale farms were involved in this study. For the purpose of this study, farms were designated as A Mazimbu University Farm, B—SUA Model Dairy Farm, C—Animal Research Unit, D—Livestock Training Agency Farm, E—Prison-owned Dairy Farm, and F—Pangawe Dairy Farm. Among the study farms, five farms were from parastatal organizations (A to E), and one farm (F) was a privately owned dairy farm. Farms were selected based on the criteria of keeping crossbreed dairy cows (*Bos taurus* x Zebu), having an average of 20 to 50 female cows in milking, and acceptance of being part of the study. In farms A and B, animals were grazed on a mixture of established and natural pastures. While animals in other farms (C, D, E and F) were allowed to graze on natural pastures. Lactating cows were either machinery-milked (farms A and B) or hand-milked (farms C, D, E and F) twice daily.

Before milking, all farms used to clean the animals' teats/udder with warm water. All farms which practiced hand-milking, milking jelly was routinely used. Neither of the farms had mastitis pre-testing nor post-milking teat dip practices. Except for farm F where calf sucking after milking was practiced, other farms used bucket feeding to calves.

Study Design and Sample Size Estimation

A cross-sectional study was conducted from July to October 2024 to determine the risk factors and prevalence of subclinical mastitis. A structured questionnaire survey was used to capture the risk factors associated with the occurrence of mastitis at farms. During the questionnaire inquiry, information regarding the animal's age, breed parity and lactation stage; milking method used; hygienic measures taken before, during and after milking; routine screening for mastitis; drying-off methods used; and floor type was gathered by questioning farm workers and owners. Mastitis prevalence was assessed using three tests: Sodium Lauryl Sulfate (SLS), California Mastitis Test (CMT), and Somatic Cell Count (SCC). The sample size was calculated according to Akoglu **(2022),** based on dependent paired diagnostic accuracies of the tests (SLS Test, CMT and SCC) employed for comparing the sensitivity or specificity. The computed sample size for the study was 186 lactating cows of different age groups, parities and lactation stages. However, animals in the first week of lactation with colostrum milk were excluded.

Clinical Inspection of Udder, Sample Collection and Screening for Subclinical Mastitis

Investigation for any abnormalities in secretions and udder (size, consistency and temperature) in all the lactating cows were assessed as per the guidelines outline elsewhere **(Quinn *et al*., 2004; Böker *et al*., 2023).** To ensure cleanliness, teats, and udders were washed, dried, and treated with 70% ethanol before sample collection. Three streams of milk were discarded. Two mL of milk was collected from each quarter using a paddle for California Mastitis reagent and SLS tests for subclinical mastitis as cow-side fast screening methods. Thereafter, approximately 15 mL of milk was collected from each quarter in a sterilized falcon tube and kept in a cooler box before transported to the laboratory and evaluated for Lactoscan SCC within the same day **(Alhussien *et al*., 2021)**.

Mastitis screening using the California Mastitis Test

On-farm screening for subclinical mastitis was performed using CMT **(Tommasoni *et al*., 2023).** Briefly, 2 ml of milk sample was mixed with equal quantity of CMT reagent in each cup of CMT paddle and rotated for few seconds and results were read and scored visually within 30 seconds for gel formation. The results were recorded as negative (no gel formation), trace (±), weak reaction (+), moderate reaction (++), and strong reaction (+++). In this study, all milk samples giving weak to strong reactions were considered as positive for SCM.

Mastitis Screening Using Sodium Lauryl Sulfate

Liquid 3% Sodium lauryl Sulfate (SLS) test solution was prepared according to **(Thakur *et al*., 2018)**. Sample testing and results were recorded as per CMT testing.

Measurement of Somatic Cells Count

Somatic cell count was measured in raw milk samples using the Lactoscan Somatic Cell Counter (Milkotronic Ltd., Bulgaria) device and in accordance with the manufacturer's instructions **(Milkotronic, 2017)**. A threshold of 200,000 cells/mL was used as discrimination for intramammary infections, whereas milk samples were considered positive only when the SCC value was ≥200,000 mL^{-1}, and milk samples were considered infected with mastitis or not based on the SCC interpretation presented in **Table 1** as previously described **(Fosgate *et al*., 2013; Narváez-Semanate *et al.*, 2022)**.

Table 1: Somatic cell counts range and interpretation

S/N	Result symbol	Interpretation	Equivalent SCC range (cell/ml)	Remarks
1	-	Negative	0 – 200,000	Healthy mammary quarter
2	±	Trace	> 200,000 – 400, 000	Possibility of having mastitis
3	+	Weak positive	400,000 – 1,200,000	Subclinical mastitis infection (Grade 1 mastitis)
4	++	Distinct positive	1,200,000 -5,000,000	Serious mastitis (Grade 2 mastitis)
5	+++	Strong positive	Over 5,000,000	Serious mastitis infection (Grade 3 mastitis)

Data analysis

Statistical analysis used SPSS software for Prevalence, Sensitivity, Specificity, and Positive and Negative Predictive Values for each diagnostic test were computed. Agreements of the screening tests were computed using Kappa statistics and interpretation of the test agreement used the Cochran Kappa Interpretation table. Moreover, Receiver Operating Characteristic (ROC) curves were sketched and AUC was computed to compare the performance accuracy of each test **(Monaghan *et al*., 2021; Tanni *et al*., 2021a).**

RESULTS

A total of 742 milk samples from 186 crossbred dairy cows were subjected to CMT, SLS, and SCC screening tests. The study revealed that most of the farms were using hand milking (66.7%, n=4), and a minority (33.3%, n=2) were practicing machine milking **(Table 2).** All farms washed animals' udders and teats before milking; no farm practiced milk pre-testing and post-dipping after milking. Dry cow therapy was practiced on one farm (16.7%), while the majority of farms (83.3%; n=5) did not apply dry cow therapy. Routine mastitis checks were practiced by two farms (33.3%), while the other four farms (66.7%) had no routine check for mastitis.

Table 2: Characteristics and milking hygienic practices in the study farms

Farm	Hygiene Practices before, during and after milking	Animal housing and management	Types of milking	Regular screening of mastitis	Milking mastitic cow at the end	Use of long acting antibiotic during drying-off period
A	Washing udder before milking and use of milk salve	Concrete floor with regular cleaning of milking parlor, and daily cleaning of concrete animal houses	Machine	Yes	Yes	Sometimes
B	Washing udder before milking	Concrete floor with regular cleaning of milking parlor, and infrequent cleaning of concrete animal house	Machine	No	No	No
C	Washing udder before milking, and use of milk salve	Concrete floor with regular cleaning of milking parlor, and infrequent cleaning of concrete animal house	Hand	Yes	Yes	Sometimes
D	Washing udder before milking and use milk salve	Concrete floor with regular cleaning of milking parlor, and daily cleaning of concrete animal house	Hand	Yes	Yes	Sometimes
E	Washing udder before milking, and use milk salve	Concrete floor, regular cleaning of milking parlor but, infrequent cleaning of earthen animal house	Hand	Yes	Yes	No
F	Washing udder before milking, and use of milk salve	Concrete floor with regular cleaning of milking parlor but, infrequent cleaning of earthen animal house	Hand	Yes	Yes	Yes

Farm-based prevalence of Mastitis is shown on **Table 3** and **Fig. 1**. The prevalence of subclinical mastitis varied significantly among the farms ($p<0.0001$) with Farm B having the highest prevalence **(Fig. 1).** The status of subclinical mastitis indicated that Farm B had the highest prevalence of subclinical mastitis with both SLS and CMT scores (82.6% n=19) also the somatic cell count was high (65.2% n=15). Farm F had the lowest prevalence (16% n=4) for both SLS and CMT and the SCC was low (10.7% n=3).

Table 3: Status of Subclinical mastitis in Lactating cows detected by SLS, CMT and SCC

Farm name	No Lactating animals	Number (%) of subclinical mastitis		
		SLS	CMT	SCC
A	28	10 (35.71)	10 (35.71)	04 (14.29)
B	23	19 (82.61)	19 (82.61)	15 (65.22)
C	17	07 (41.18)	07 (41.18)	02 (11.77)
D	28	08 (28.57)	08 (28.57)	04 (14.29)
E	65	20 (30.77)	20 (30.77)	12 (18.46)
F	25	04 (16.00)	04 (16.00)	03 (12.00)
TOTAL	186	68 (36.56)	68 (36.56)	40 (21.51)

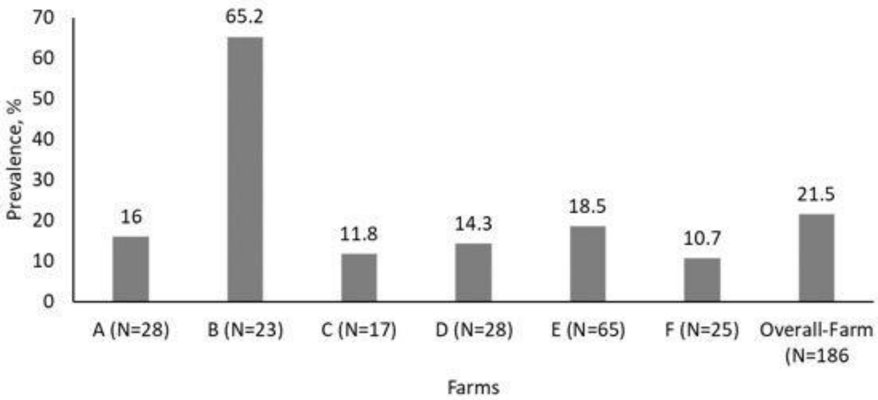

Fig. 1: Farm-based prevalence of Mastitis

Figure 2 shows the prevalence of mastitis from 742 milk samples collected from 186 lactating cows. Two out of 744 (0.27%) mammary quarters were blind and hence were not considered for the prevalence of SCM detection. SLS detected 16.9% (126 out of 742) of mammary quarters to be affected by SCM, while CMT picked 15.8% (117 out of 742) to have subclinical mastitis. No statistical difference (p=0.57) was noted between the two tests. SCC revealed a low number of quarters with prevalence (8.5%; n=63) that were positive to SCM. However, a significant difference (p<0.0001) was observed when SCC was compared with the other two tests (CMT and SLS). Quarter-side analysis of subclinical mastitis revealed the right hind quarter to have the highest prevalence (19.5%, n=36) for both SLS and CMT, while SCC picked the fore-left quarter to be more affected (10.22%) than the other quarters (**Table 4**).

Table 4: Quarter-side subclinical mastitis testing results based on SLS, CMT, and SCC.

Quarter side	Number Lactating Quarters	Number of samples	Number (%) of Subclinical Mastitis		
			SLS	CMT	SCC
Fore-Right	186	186	29 (15.59)	27 (14.52)	14 (7.53)
Fore-Left	186	186	33 (17.74)	30 (16.13)	19 (10.22)
Hind-Left	186	185*	26 (14.60)	24 (12.97)	14 (7.57)
Hind-Right	186	185*	36 (19.46)	36 (19.46)	16 (8.64)
TOTAL	744	742	126 (16.85)	117 (15.77)	63 (8.49)

*Blind mammary gland quarters (1 Hind-Left and 1 Hind-Right).

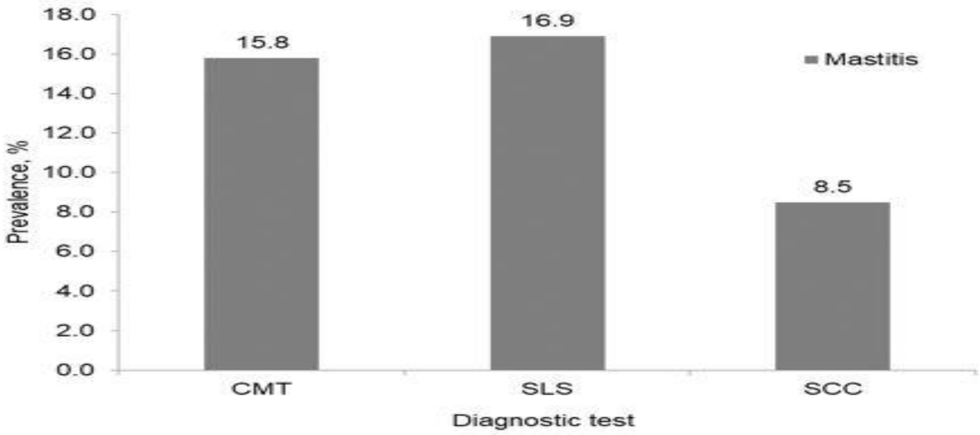

Fig. 2: Prevalence of subclinical mastitis based on CMT, SLS Test, and SCCs diagnostic methods

In assessing the sensitivity and specificity of SLS and CMT compared with the standard gold test used in this study (SCC), various data were observed **(Figs. 3 and 4).** Of the 63 milk samples that initially tested positive for SLS, were also found positive in SCC. 57 out of 63 (90.48%) samples that were positive by CMT were also positive for SCC, while 6 (9.52%) were reconfirmed as negative (false positive) by SCC. 63 (9.28%) and 616 (90.72%) out of 679 samples initially recorded negative (health quarters) through SLS testing were recategorized as positive (false positive) and negative (true negative), respectively, when challenged to SCC. Similarly, 60 out of 679 (8.84%) samples initially categorized as negative through CMT screening were reconfirmed as positive (false positive) through SCC (95% CI=6.1-10.0), while 619 out of 679 (91.16%) were reconfirmed as negative (true negative) by SCC testing (95% CI=80.7-86.1). Fagan's monogram test for the positive likelihood ratio for the SLS test (LR+ = 10.78) was marginally better than the CMT test (LR+ = 10.24) in confirming subclinical mastitis after a positive result. This means that after a cow tested positive on the SLS test, the post-test probability of having subclinical mastitis was slightly higher than the CMT test. In the screening for subclinical mastitis in lactating cows, the SLS test, with Fagan's nomogram negative likelihood ratio (LR- = 0), indicated that a negative result effectively ruled out the presence of subclinical mastitis with complete certainty. In contrast, the CMT test, with an LR- = 0.10, suggested that a negative result reduced the likelihood of subclinical mastitis by 90%, though some residual risk remained. While both tests lowered the probability of disease, the SLS test demonstrated superior diagnostic accuracy in excluding subclinical mastitis compared to the CMT test.

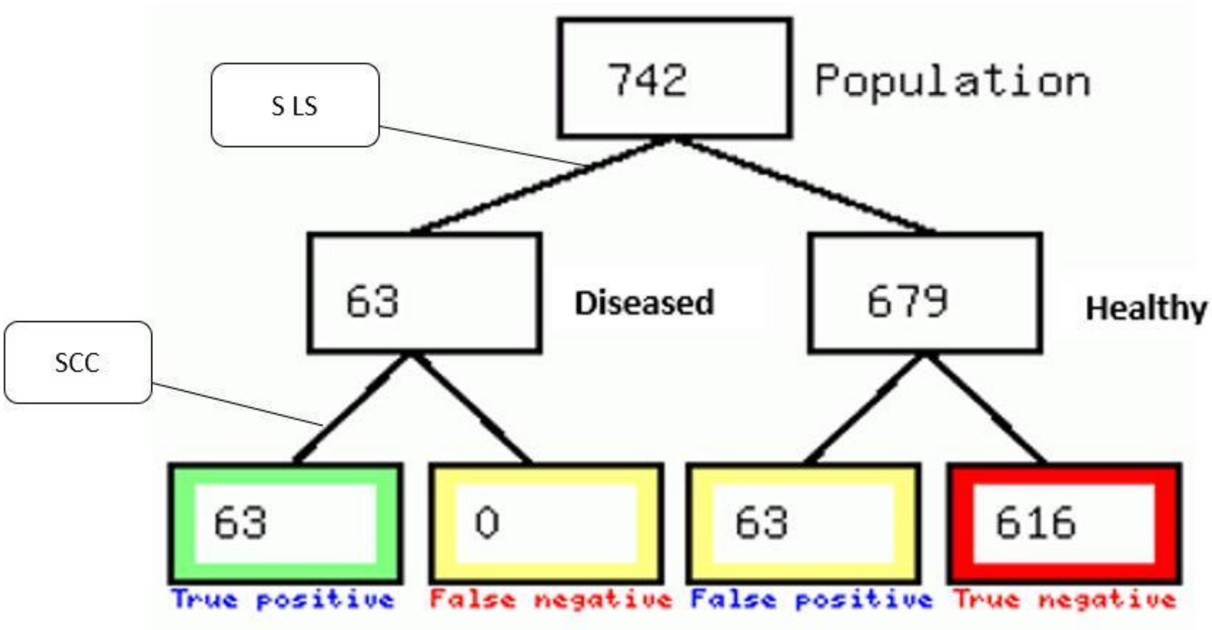

Fig. 3: SLS Test against SCC

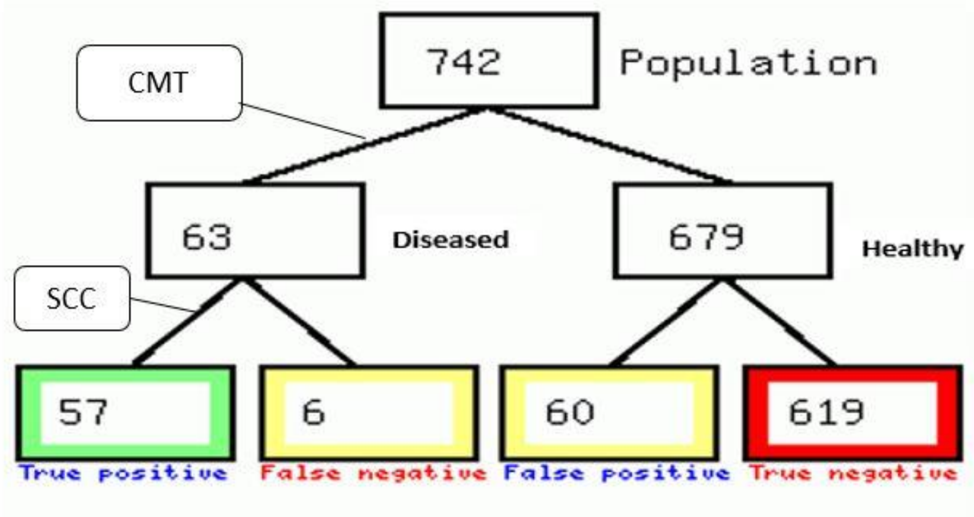

Fig. 4: CMT against SLS

When a comparison test was performed to assess the efficacy of SLS and CMT about SCC, the SLS test demonstrated perfect sensitivity (100%) and a high negative predictive value (NPV) of 100%, indicating it reliably detected all cases of mastitis and effectively ruled out healthy cows.

However, CMT had slightly lower sensitivity (90.48%) and NPV (99.04%), suggesting it might miss some cases. Both SLS and CMT showed high specificity values: 91.16% for CMT and 90.72% for the SLS **(Table 5).** Nevertheless, both exhibited low positive predictive values (around 50%), highlighting a significant rate of false positives when evaluating diagnostic performance; the SLS test had a higher likelihood ratio for positive results (10.78) compared to the CMT (10.24), making it more effective in diagnosing mastitis when the test is positive. The CMT provided a defined diagnostic odds ratio (98.01), while the SLS Test's odds remained undefined due to its perfect sensitivity. Cohen's kappa values indicated moderate agreement with the SCC, with the SLS Test slightly ahead of CMT (0.62 vs. 0.58). Overall, the SLS test excelled in sensitivity and NPV when aligned with SCC, whereas the CMT offered slight advantages in specificity and diagnostic odds, making both tests valuable in different contexts. When assessing the diagnostic efficiency of SLS and CMT, the Receiver Operator Characteristic (ROC) Curve of CMT (0.997) and SLS (0.996) were almost similar, showing the same diagnostic efficiency.

Table 5: Diagnostic performance of SLS and CMT tests when compared to SCC.

Parameter	Diagnostic performance	
	SLS	CMT
Sensitivity, %	100 (94.25-100)	90.48 (80.74-95.56)
Specificity, %	90.72 (88.3-92.68)	91.16 (88.79-93.07)
Positive Predictive Value (PPV), %	50 (41.4-58.6)	48.72 (39.85-57.67)
Negative Predictive Value (NPV), %	100 (99.38-100)	99.04 (97.92-99.56)
Diagnostic Accuracy, %	91.51 (89.28-93.31)	91.11 (88.84-92.95)
Likelihood Ratio of a Positive Test,	10.78 (10.45-11.12)	10.24 (9.87-10.62)
Likelihood Ratio of a Negative Test	0	0.10 (0.075 - 0.14)
Diagnostic Odds	Undefined	98.01(40.57-236.8)
Cohen's Kappa (Unweighted)	0.62 (0.55-0.69)	0.58 (0.5201-0.65)
Bias Index	0.08491	0.07278

DISCUSSION

Mastitis is a serious lactation-related disease in dairy cattle farms causing significant economic losses in the quantity and quality of milk globally. The overall prevalence (21.5%) of subclinical mastitis reported in the present study is comparable with 21.7% reported in smallholder dairy farms in Mvomero and Njombe district, Tanzania **(Mdegela *et al.*, 2009)**. However, our result is lower than the 44.55% reported in small-scale farmers in Dodoma, Tanzania **(Mramba and Mohamed, 2024)**, 65% subclinical mastitis prevalence in dairy cattle from institution farms in Morogoro Municipality **(Mgonja *et al***., **2023)**, 34-51% in dairy cows in Kenya **(Ngotho *et al*., 2022)**, 43.1 to 76.2% in dairy herds in Rwanda **(Ndahetuye *et al*., 2019)** and subclinical mastitis ranges from 61.3% to 87.9% in Ugandan dairy cows **(Abrahmsén *et al*., 2014; Kakooza *et al*., 2023)**. The quarter-level frequency of subclinical mastitis reported in this study (15.8%, 16.9%, and 8.5% for CMT, SLS, and SCC, respectively) was comparable to the 8.3% to 12.3% CMT prevalence report in Ethiopia **(Belachew, 2016)**, but lower than 63.7%, 78.8%, and 65% for CMS, SLS, and SCC, respectively, that was reported by other researchers **(Damian *et al*., 2021; Biscotto *et al.*, 2022; Mgonja *et al*., 2023)**. Variations in mastitis prevalence might be attributed to many factors, including farm management systems (intensive versus extensive), breeds of cows, agroecology of the study sites, milking practices, hygiene of the cow shed, and farm disease control program **(Mureithi and Njuguna, 2016)**.

Analysis of variance revealed that the two screening tests (SLS; 16.85% and CMT; 16.85%) had no significant difference in overall quarterly subclinical prevalence results except for the SCC test (8.49%). This is parallel to the result of SLS and CMT reported elsewhere **(Waminal *et al*., 2021; Gangan, 2023)**. The low SCC prevalence may have been caused by the cut-off value (200,000 cells/ml). Somatic cells less than 200,000 cells/ml were interpreted as negative subclinical mastitis, and above 200,000 SSC/ml were considered positive subclinical mastitis **(Sargeant *et al*., 2001; Huang and Kusaba, 2022)**. Due to their subjectivity, the CMT and possibly SLS tests require a visual interpretation of the milk changes, which can be influenced by human error or variability in judgment. This makes the results less consistent than objective tests like SCC measurements **(Sargeant *et al.*, 2001; Ruegg and Reinemann, 2002; Huang and Kusaba, 2022)**. The observed prevalence of 82.6% for subclinical mastitis at farm B was high. Similar findings have been reported in Ethiopia, where a prevalence rate of 82.6% has been documented in smallholder farms by **Zeryehun *et al*., (2013)**. Such high rates are often linked to poor milking hygiene, inadequate mastitis control programs, and a lack of awareness among farmers **(Mdegela *et al.*, 2004)**. A study in Kibaha, Tanzania, reported a subclinical mastitis prevalence of 75%, underscoring the widespread nature of the disease **(Kivaria, 2006).** The relatively high occurrence of subclinical mastitis on farm B could be correlated to milking inefficiency and limited control measures undertaken at the farm, which are critical control points in preventing the spread of mastitis **(Mdegela *et al.*, 2004)**.

In contrast, farm F showed a lower prevalence of subclinical mastitis at 10%, with a correspondingly low SCC in 10.7% of animals. The farm F had calves sucking milk direct from cows; this is in agreement with studies by **Köllmann *et al.*, (2021)** that calves direct suckling from cows improved udder health. This suggests that farm management practices play a pivotal role in controlling mastitis. According to **Mdegela *et al.*, (2009),** farms with better management practices, including regular monitoring of udder health, proper sanitation, and effective mastitis control strategies, often had lower prevalence rates. Better management practices lead to an increase in milk production and milk quality, and thus an increase in the profitability of the enterprise **(Çýlek and Tekýn, 2007; Çilek and Gotoh, 2012)**. The right hind quarter had the highest prevalence of mastitis for both ALS and CMT (19.4%, n=36), while the reference standard SCC showed the lowest on the fore left quarter, reported at 7.5% (n=14) (Table 3). The hindquarter teats are longer and have more milk than the forequarter teats. The higher prevalence of hindquarters teats to subclinical mastitis is likely attributed to the anatomical size of hind teats, which are longer and have a larger volume of milk than the forequarter teats, with higher risks of contamination than forequarter teats **(Damian *et al*., 2021)**.

The sensitivity and specificity of the SLS were 100% and 90.72%, respectively. In contrast, the CMT had a lower sensitivity of 90.48%, suggesting that it may miss some cases. Both tests showed higher specificity with CMT (91.16%) than the SLS test (90.72%). The results are in agreement with that of Tanni ***et al.* (2021)** who reported the sensitivity and specificity of SLS at 100% and 83.3%, respectively. However, the CMT sensitivity and specificity values are above those reported elsewhere **(Dingwell *et al*., 2003; Leach *et al*., 2008; Reddy *et al.*, 2014)**. Variation of the CMT sensitivity and specificity may be due to the subjective interpretation of milk changes as stipulated earlier **(Sargeant *et al*., 2001; Ruegg and Reinemann, 2002; Leach *et al*., 2008; Huang and Kusaba, 2022)**. The high sensitivity of the SLS test indicated the proportion of infected quarters that were correctly identified as infected. Additionally, the specificity of the SLS solutions was observed to be high, indicating its effectiveness in correctly identifying uninfected samples. These favorable intrinsic properties of the SLS solutions enhance its suitability as a reliable diagnostic tool for detecting subclinical mastitis (SCM) in dairy cows. The findings are in agreement with study results by **Tanni *et al*., (2021a)** that showed the sensitivity of SLS at 100%.

Positive Predictive Value of the SLS Test and CMT was reported at 50% and 48.7%, respectively. The SLS Test and CMT Negative Predictive obtained were reported at 100% and 99.04%, respectively, which are higher than the study of **Sharma and Pandey (2011),** who reported a positive Predictive Value of 57.3% and a Negative Predictive Value of 30.6%, and **Dingwell *et al*., (2003)** who reported CMT PPV at 21.1% and 95.5%, respectively. False positives may have been attributed to positive and negative predictive value variations. Cohen's Kappa for SLS was 0.62 and CMT was 0.58, showing that the SLS Test and CMT had a moderate agreement with the preference standard, which is SCC. The results are consistent with the results by **Tanni *et al*., (2021a)** who reported Cohen's Kappa at 0.615 and 0.455 on the SLS Test and CMT, respectively. The area under the Receiver Operating Characteristic (ROC) Curve for the SLS and CMT was 0.996 and 0.997 with differences of 0.001. **Tanni *et al*., (2021a)** reported the ROC Curve of SLS at 0.917, which is lower than the current ROC Curve. Also, the studies conducted by **Ali and Dahl (2022)** reported the ROC Curve for CMT at 0.801, which is smaller than the ROC Curve of the current study.

The differences might be caused by subjective evaluation of the milk and the different animal species used (water buffaloes). Despite differences in their curve of studies, the larger the ROC curve, the better the diagnostic efficiency of the test (Hajian-Tilaki, 2013). The current findings on likelihood ratios of SLS and CMT, which were LR+ = 10.78 and LR+ = 10.24, are in agreement with the findings by **Jacobsen *et al*., (2023)** who reported LR+ = 10 as the best likelihood of the diagnostic test in confirming SCC in subclinical mastitis and LR- = 0 and LR- = 0.10 for SLS and CMT for ruling out the SSC in subclinical mastitis by 100% and 90%, respectively.

CONCLUSION

High SCM prevalence in some herds involved in this study might be attributed to poor housing, poor hygienic conditions, bad milking practices, lack of regular screening and prompt treatment of diseased animals, and likely contaminated milking machines. Thus, awareness creation on regular testing for subclinical mastitis, improvement of milking hygiene, and udder health management practices should be incorporated in the farms in order to minimize the burden of subclinical mastitis. Furthermore, when comparing the efficiency of detecting subclinical mastitis using CMT and the SLS substance, there were no significant differences in results. Thus, we provide a road map for using SLS tests as an alternative to commercially available CMT for detecting subclinical mastitis in field conditions, as the SLS is cheaper in terms of cost of preparation and use, and it is more readily available in the market. However, future studies should focus on conducting studies to evaluate the shelf life and stability of the SLT solution under field conditions in developing tropical countries.

Conflict of interest

The authors declare no conflicts of interest related to the content of this article.

Acknowledgment

We would like to express our heartfelt gratitude to Nickson Kinyamagoha, for his technical assistance in Lactoscan SCC laboratory work. The authors are indebted to the respective farm managers for giving us permission to use their animals during this study and giving their time during the face-to-face interview.

REFERENCES

ABRAHMSÉN, M., PERSSON, Y., KANYIMA, B. M., and BÅGE, R., 2014. Prevalence of subclinical mastitis in dairy farms in urban and peri-urban areas of Kampala, Uganda. Tropical Animal Health and Production, 46(1), 99–105. https://doi.org/10.1007/s11250-013-0455-7

AKOGLU, H. 2022. User's guide to sample size estimation. 177–185. https://doi.org/10.4103/2452-2473.357348

ALHUSSIEN, M. N., and DANG, A. K., 2018. Milk somatic cells, factors influencing their release, future prospects, and practical utility in dairy animals: An overview. Veterinary World, 11(5), 562–577. https://doi.org/10.14202/vetworld.2018.562-577

ALHUSSIEN, M. N., PANDA, B. S. K., and DANG, A. K., 2021. A Comparative Study on Changes in Total and Differential Milk Cell Counts, Activity, and Expression of Milk Phagocytes of Healthy and Mastitic Indigenous Sahiwal Cows. Frontiers in Veterinary Science, 8(June), 1–12. https://doi.org/10.3389/fvets.2021.670811

ALI, H. W., and DAHL, M. O., 2022. Comparison of California mastitis test and Draminski mastitis detector as on-farm methods for monitoring udder health in lactating buffalo. Iraqi Journal of Veterinary Sciences, 36(supp1), 55–60. https://doi.org/10.33899/ijvs.2022.135362.2469

ANDREWS, A. H. 2004. Bovine Medicine Diseases and Husbandry of Cattle (R. W. B. H. B. R. G. Eddy (ed.); Second edi, Vol. 2). Blackwell Publishing Company. https://doi.org/10.1002/9780470752401.ch14

BAEZA, C. 2016. Acute, Subclinical, and Subacute Mastitis. Clinical Lactation, 7(1), 7–10. https://doi.org/10.1891/2158-0782.7.1.7

BELACHEW, T. 2016. Bovine Mastitis: Prevalence, Isolation of Bacterial Species Involved and its Antimicrobial Susceptibility Test around Debrezeit, Ethiopia. Journal of Veterinary Science and Technology, 07(06). https://doi.org/10.4172/2157-7579.1000396

BISCOTTO, J. V. G., AMARAL, M. M., and DA CUNHA, A. F., 2022. Prevalence and influence of clinical and subclinical mastitis in the service period of dairy cows in Tarumirim (MG). Acta Veterinaria Brasilica, 16(3), 205–210. https://doi.org/10.21708/avb.2022.16.3.10609

BÖKER, A. R., BARTEL, A., DO DUC, P., HENTZSCH, A., REICHMANN, F., MERLE, R., ARNDT, H., DACHRODT, L., WOUDSTRA, S., and HOEDEMAKER, M., 2023. Status of udder health performance indicators and implementation of on farm monitoring on German dairy cow farms: results from a large scale cross-sectional study. Frontiers in Veterinary Science, 10(May). https://doi.org/10.3389/fvets.2023.1193301

ÇILEK, S., and GOTOH, T., 2012. Effects of calving years, times and seasons on milk yield traits in Turkish brown Swiss cows in a steppe climate. Journal of the Faculty of Agriculture, Kyushu University, 57(2), 447–451. https://doi.org/10.5109/25204

COBIRKA, M., TANCIN, V., and SLAMA, P. 2020. Epidemiology and Classification of Mastitis. 1–17.

ÇÝLEK, S., and TEKÝN, M. E., 2005. Environmental factors affecting milk yield traits of Brown Swiss cows raised at Ulaş State Farm and phenotypic correlations between milk yield and fertility traits. Indian Journal of Animal Sciences, 77(2), 154–157.

DAMIAN, K., ROBINSON, M., LUGHANO, K., and GABRIEL, S., 2021. Prevalence and risk factors associated with subclinical mastitis in lactating dairy cows under smallholder dairy farming in North East Tanzania. Journal of Veterinary Medicine and Animal Health, 13(1), 55–64. https://doi.org/10.5897/jvmah2019.0775

DINGWELL, R. T., LESLIE, K. E., SCHUKKEN, Y. H., SARGEANT, J. M., and TIMMS, L. L., 2003. Evaluation of the California mastitis test to detect an intramammary infection with a major pathogen in early lactation dairy cows. Canadian Veterinary Journal, 44(5), 413–416. https://pmc.ncbi.nlm.nih.gov/articles/PMC340150/

DOHOO, I. R., SMITH, J., ANDERSEN, S., KELTON, D. F., and GODDEN, S., 2011. Diagnosing intramammary infections: Evaluation of definitions based on a single milk sample. Journal of Dairy Science, 94(1), 250–261. https://doi.org/10.3168/jds.2010-3559

FOSGATE, G. T., PETZER, I. M., and KARZIS, J., 2013. Sensitivity and specificity of a hand-held milk electrical conductivity meter compared to the California mastitis test for mastitis in dairy cattle. Veterinary Journal, 196(1), 98–102. https://doi.org/10.1016/j.tvjl.2012.07.026

GANGAN, J. E. 2023. Low- Cost Liduid Anionic Surfactants in Mastitis Detection for Small-Hold Dairy Buffalo Farmers. July.

GODDEN, S. M., ROYSTER, E., TIMMERMAN, J., RAPNICKI, P., and GREEN, H., 2017. Evaluation of an automated milk leukocyte differential test and the California Mastitis Test for detecting intramammary infection in early- and late-lactation quarters and cows. Journal of Dairy Science, 100(8), 6527–6544. https://doi.org/10.3168/jds.2017-12548

HAJIAN-TILAKI, K. 2013. Receiver operating characteristic (ROC) curve analysis for medical diagnostic test evaluation. Caspian Journal of Internal Medicine, 4(2), 627–635.

HUANG, C. H., and KUSABA, N., 2022. Association between differential somatic cell count and California Mastitis Test results in Holstein cattle. JDS Communications, 3(6), 441–445. https://doi.org/10.3168/jdsc.2022-0249

JACOBSEN, L. A., NIESEN, A. M., LUCEY, P., and ROSSOW, H. A., 2023. Evaluation of Cow-Side Meters to Determine Somatic Cell Count in Individual Cow Quarter and Bulk-Tank Milk Samples. Animals, 13(13). https://doi.org/10.3390/ani13132169

KAKOOZA, S., MUTEBI, F., SSAJJAKAMBWE, P., WAMPANDE, E., NABATTA, E., ATUHEIRE, C., TSUCHIDA, S., OKUBO, T., USHIDA, K., and KANEENE, J. B., 2023. Mastitis on selected farms in Wakiso district, Uganda: Burden, pathogens and predictors of infectivity of antimicrobial resistant bacteria in dairy herds. Veterinary Medicine and Science, 9(5), 2376–2385. https://doi.org/10.1002/vms3.1234

KIVARIA, F. M. 2006. Epidemiological Studies on Bovine Mastitis in Smallholder Dairy Herds in the Dar es Salaam Region , Tanzania. Tropical Animal Health and Production, 169.

KÖLLMANN, K., ZHANG, Y., WENTE, N., LÜCKEN, A., LEIMBACH, S., and KRÖMKER, V., 2021. Effects of Suckling on the Udder Health of Foster Cows. Ruminants, 1(2), 100–117. https://doi.org/10.3390/ruminants1020008

KOMBA, E. V., and KASHOMA, I. P., 2020. Prevalence and Economic Impacts of Retained Placenta and Subclinical Mastitis in Tanzanian Dairy Cows. 1–25.

KOUR, S., SHARMA, N., BALAJI, N., KUMAR, P., SOODAN, J. S., VEIGA, M., and SON, Y., 2023. veterinary sciences Advances in Diagnostic Approaches and Therapeutic Management in Bovine Mastitis.

LEACH, K. A., GREEN, M. J., BREEN, J. E., HUXLEY, J. N., MACAULAY, R., NEWTON, H. T., and BRADLEY, A. J., 2008. Use of domestic detergents in the California mastitis test for high somatic cell counts in milk. Veterinary Record, 163(19), 566–570. https://doi.org/10.1136/vr.163.19.566

MDEGELA, R. H., KUSILUKA, L. J. M., KAPAGA, A. M., KARIMURIBO, E. D., TURUKA, F. M., BUNDALA, A., KIVARIA, F., KABULA, B., MANJURANO, A., LOKEN, T., and KAMBARAGE, D. M., 2004. Prevalence and determinants of mastitis and milk-borne zoonoses in smallholder dairy farming sector in Kibaha and Morogoro districts in eastern Tanzania. Journal of Veterinary Medicine Series B: Infectious Diseases and Veterinary Public Health, 51(3), 123–128. https://doi.org/10.1111/j.1439-0450.2004.00735.x

MDEGELA, R. H., RYOBA, R., KARIMURIBO, E. D., PHIRI, E. J., LØKEN, T., REKSEN, O., MTENGETI, E., and URIO, N. A., 2009. Prevalence of clinical and subclinical mastitis and quality of milk on smallholder dairy farms in Tanzania. Journal of the South African Veterinary Association, *80*(3), 163–168. https://doi.org/10.4102/jsava.v80i3.195

MESA, C. LA, and RISULEO, G. 2021. Surface Activity and Ef fi ciency of Cat-Anionic Surfactant Mixtures. 9(December), 1–9. https://doi.org/10.3389/fchem.2021.790873

MGONJA, F. R., CHARLES, M. M., and KATAKWEBA, A. S., 2023. Article 1019 Citation: Mgonja FR, Charles MM, Katakweba AS. Prevalence of Subclinical Mastitis and Associated Risk Factors in Dairy Cattle from Institution Farms in Morogoro Municipality. World J Vet Sci, 4(1), 1020.

MILKOTRONIC, 2017. LACTOSCAN SCC Operation Manual.127.http://lactoscan.com/editor/ufo/manuals/SCC/IM_SCC_EN.pdf

MONAGHAN, T. F., RAHMAN, S. N., AGUDELO, C. W., WEIN, A. J., LAZAR, J. M., EVERAERT, K., and DMOCHOWSKI, R. R., 2021. Foundational statistical principles in medical research : Predictive value. Medicina, 57(5), 503.

MRAMBA, R. P., and MOHAMED, M. A., 2024. The prevalence and factors associated with mastitis in dairy cows kept by small-scale farmers in Dodoma, Tanzania. Heliyon, 10(13), e34122. https://doi.org/10.1016/j.heliyon.2024.e34122

MUREITHI, D. K., and NJUGUNA, M. N., 2016. Prevalence of subclinical mastitis and associated risk factors in dairy farms in urban and peri-urban areas of Thika Sub County, Kenya. Livestock Research for Rural Development, 28(2).

NARVÁEZ-SEMANATE, J. L., DAZA-BOLAÑOS, C. A., VALENCIA-HOYOS, C. E., HURTADO-GARZÓN, D. T., and ACOSTA-JURADO, D. C., 2022. Diagnostic methods of subclinical mastitis in bovine milk: an overview. Revista Facultad Nacional de Agronomia Medellin, 75(3), 10077–10088. https://doi.org/10.15446/rfnam.v75n3.100520

NDAHETUYE, J. B., PERSSON, Y., NYMAN, A. K., TUKEI, M., ONGOL, M. P., and BÅGE, R., 2019. Aetiology and prevalence of subclinical mastitis in dairy herds in peri-urban areas of Kigali in Rwanda. Tropical Animal Health and Production, 51(7), 2037–2044. https://doi.org/10.1007/s11250-019-01905-2

NGOTHO, M., KAGIRA, J., NKOIBONI, D., NJOROGE, J., and MAINA, N., 2022. Risk Factors Associated with Sub-Clinical Mastitis and Antibacterial Resistance in Small-Holder Dairy Farms of Kajiado North Sub-County, Kenya. Journal of Veterinary Physiology and Pathology, 1(3), 49–55. https://doi.org/10.58803/jvpp.v1i3.8

QUINN, P.J., CARTER, M. E., MARKEY, B., and CARTER, G.R., 2004. clinical veterinary microbiology - Google Search (p. 646). Elsevier Limited.https://www.google.com.np/search?tbm=bksandhl=enandq=clinical+veterinary+microbiology

REDDY, B. S. S., KUMARI, K. N., REDDY, Y. R., REDDY, M. VIJAYA B., and REDDY, B. S., 2014. International Journal of Veterinary Science Comparison of Different Diagnostic Tests in Subclinical Mastitis in Dairy Cattle. International Journal of Veterinary Science, 3(4), 224–228. http://www.ijvets.com/pdf-files/Volume-3-no-4-2014/224-228.pdf

RUEGG, P. L., and REINEMANN, D. J., 2002. Milk quality and mastitis tests. The Bovine Practitioner, 41–54. https://doi.org/10.21423/bovine-vol36no1p41-54

SARGEANT, J. M., LESLIE, K. E., SHIRLEY, J. E., PULKRABEK, B. J., and LIM, G. H., 2001. Sensitivity and specificity of somatic cell count and California Mastitis Test for identifying intramammary infection in early lactation. Journal of Dairy Science, 84(9), 2018–2024. https://doi.org/10.3168/jds.S0022-0302(01)74645-0

SHARIF, A., and MUHAMMAD, G. 2009. Mastitis control in dairy animals. Pakistan Veterinary Journal, 29(3), 145–148. https://www.researchgate.net/publication/286019716_Mastitis_control_in_dairy_animals#fullTextFileContent

SHARMA, N., and PANDEY, V., 2011. Comparative evaluation of three tests used for the screening of mastitis. Indian Journal of Animal Sciences, 81(2), 140.

TANNI, N. S., ISLAM, M. S., KABIR, M., PARVIN, S., EHSAN, M. A., and ISLAM, M. T., 2021a. Evaluation of sodium lauryl sulfate for the development of cow-side mastitis screening test. Veterinary World, 14(8), 2290–2295. https://doi.org/10.14202/vetworld.2021.2290-2295

TANNI, N. S., ISLAM, M. S., KABIR, M., PARVIN, S., EHSAN, M. A., and ISLAM, M. T., 2021b. Mastitis can be caused by either infectious or non-infectious factors. Infectious agents encompass microbes, fungi, yeasts, and viruses. Bacterial infections, notably Staphylococcus aureus and Streptococcus species, are the predominant agents (Thakur et a. Veterinary World, 14(8), 2290–2295. https://doi.org/10.14202/vetworld.2021.2290-2295

THAKUR, D., SINGH, R., KUMAR, A., KUMAR JHA, V., and KUMAR SINGH, P., 2018. Isolation and Identification of Major Pathogen from Clinical and Subclinical Mastitis Milk Sample and Their Antibiotic Sensitivity Evaluation. Int.J.Curr.Microbiol.App.Sci, 7, 4315–4321. http://www.ijcmas.com

TOMMASONI, C., FIORE, E., LISUZZO, A., and GIANESELLA, M., 2023. Mastitis in Dairy Cattle: On-Farm Diagnostics and Future Perspectives. Animals, 13(15), 1–15. https://doi.org/10.3390/ani13152538

WAMINAL, Y. O. 2021. Available Online at : https://www.scholarzest.com. Low- Cost Liquid Anionic Surfactant in Mastitis Detection for Small Scale Dairy Buffalo Raisers in Philippines. 2(4), 43–48.

WAMINAL, Y. O., GAMBOL, M. R., SABADO, A. C., VALLEJOS, J. P. M., BRIONES, R. C., and ESTABILLO, E. J. M., 2021. Mastitis Detection in Holstein Sahiwal Crossbred Cattle (Bos taurus) Using Different Brands and Dilution Levels of Liquid Anionic Surfactants. Animal Production, 23(32), 144–150. https://doi.org/10.20884/1.jap.2021.23.3.103

ZERYEHUN, T., AYA, T., and BAYECHA, R., 2013. Study on prevalence, bacterial pathogens and associated risk factors of bovine mastitis in small holder dairy farms in and around addis Ababa, Ethiopia. Journal of Animal and Plant Sciences, 23(1), 50–55.

How to cite this article:

Sangwa A. L., I.P. Kashoma and A.M. Lupindu, 2025. Comparative Evaluation of Some Diagnostic Tests for on-farm Screening of Subclinical Mastitis in Crossbreed Dairy Cows. Journal of Applied Veterinary Sciences, 10 (2):75-85.**https://dx.doi.org/10.21608/javs.2025.351733.1517**