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Comparative Relation of California Mastitis Test, Surf Field Mastitis Test and Modified Surf Field Mastitis Test for Detection of Sub-Clinical Mastitis in Cattle and Buffaloes

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Abstract

This study demonstrated a comprehensive comparative evaluation of three mastitis detection tests: California Mastitis Test (CMT), Modified Surf Field Mastitis Test (MSFMT), and Surf Field Mastitis Test (SFMT) in 100 lactating animals (50 crossbred cows and 50 buffaloes) from commercial dairy farms in Pakistan. Using a standardized protocol, 400 quarter milk samples were analyzed to assess test performance through both qualitative scoring and quantitative somatic cell count (SCC) measurements. Results exhibited CMT's superior diagnostic sensitivity, detecting subclinical mastitis in 67.18% of cow quarters (129/192) and 59.2% of buffalo quarters (119/199), with clear differentiation of infection severity (+1 to +3 grades). MSFMT demonstrated intermediate efficacy (55.5% in cows, 48.2% in buffaloes), while SFMT had the lowest detection rates (47.91% and 44.2% respectively). SCC analysis supported these findings, exhibiting significant (P<0.01) progressive increases from negative (253,756±8,205 cells/mL) to severe cases (939,745±78,558 cells/mL) in cows, with similar patterns in buffaloes. Particularly, our modified MSFMT formulation revealed 15.8% greater sensitivity than conventional SFMT in cows, suggesting its potential as a cost-effective alternative. Quarter-wise SCC distribution revealed no significant positional differences (P>0.05), indicating systemic rather than localized infection patterns. The research provides robust evidence supporting CMT as the gold standard field test while proposing MSFMT as a practical alternative for smallholder dairy operations in developing countries. These results have important implications for mastitis control programs aiming to minimize the substantial economic losses caused by subclinical infections in tropical dairy systems.

Key words: Mastitis, Bovine, Sub-Clinical, Somatic Cell Count, Dairy.

1. Introduction

Mastitis, an inflammatory condition of the mammary gland, poses vital economic and health challenges to the global dairy industry. It leads to low milk yield, declined milk quality, increased veterinary costs, and higher culling rates (Kossaibati & Esslemont, 1997). In Pakistan, where dairy farming contributes significantly to the national GDP, mastitis remains a critical matter, with estimated annual losses of Rs. 240 million in Punjab alone (Khan & Khan, 2006). The disease is a leading cause of premature culling, accounting for 22.5% of dairy cattle removals in Pakistan's Khyber Pakhtunkhwa Province (Samiullah et al., 2000).

Mastitis exhibits in clinical and subclinical forms, with the latter often overlooked despite its higher prevalence. For every clinical case, 16–41 sub-clinical infections may exist, increasing somatic cell counts (SCC) and impairing milk quality (Jones & Bailey, 2009). Subclinical mastitis is particularly insidious as milk appears normal, compelling diagnostic tests for detection. While bacterial culture remains the gold standard, it is unrealistic for routine farm use due to cost and technical requirements (Emanuelson et al., 1987).

To address this, rapid but low-cost indirect tests such as the California Mastitis Test (CMT) and Surf Field Mastitis Test (SFMT) have been industrialized. These tests rely on detergent-based reagents to detect raised leukocyte levels, with CMT being extensively used in developed nations (Contreras et al., 1995). However, in resource-limited countries like Pakistan, SFMT which is a 3% detergent solution test, offers a viable alternative (Muhammad et al., 1995). In spite of its affordability, SFMT's diagnostic accuracy comparative to CMT remains understudied.This study introduces a Modified Surf Field Mastitis Test (MSFMT) and compares its effectiveness with SFMT and CMT in detecting subclinical mastitis in cattle and buffaloes. Early and accurate diagnosis is critical for alleviating losses, as high SCC (>150,000 cells/mL) associates with infection risk (Swedish Dairy Association). By evaluating these tests, we aim to provide dairy farmers with accessible and cost-effective tools for mastitis management, ultimately improving milk production and animal welfare.

2. Material and Methods

2.1 Study Area and Animals

The research trial was conducted at the University Dairy Farm at University of Agriculture Faisalabad and private dairy farms in Faisalabad District, Pakistan (Muhammad et al., 2010). A total of one hundred (100) lactating animals (50 crossbred cows and 50 buffaloes) of varying parities were included in the study. The animal's selection were based on their lactation stage and health status to ensure representative sampling (Schalm et al., 1971).

2.2 Sample Collection and Preparation

Aseptic milk samples collection were made after proper udder cleaning with 70% ethanol and visual inspection for abnormalities such as clots, blood, or flakes (Doxey, 1971). Approximately 10 ml of milk sample was collected from each quarter of each and stored in sterile test tubes during morning milking (Athar et al., 2007). The samples were immediately tested for sub-clinical mastitis using three different methodologies.

2.3 Mastitis Detection Tests

2.3.1 California Mastitis Test (CMT)

The CMT was performed according to the manufacturer's instructions (Kenotest®, Belgium) (Schalm et al. 1971). Briefly, identical volumes (2.5-3.0 ml) of milk and CMT reagent were mixed in a paddle and swirled for 15-20 seconds. The reaction was recorded as negative (no change), 1+ (slight gel), 2+ (moderate gel), or 3+ (strong gel formation).

2.3.2 Surf Field Mastitis Test (SFMT)

The SFMT was prepared as a 3% Surf Excel® solution (3gram detergent in 100 ml water) (Muhammad et al. 1995). Two (2) ml of milk was mixed with 2 ml of SFMT solution in a test cup and swirled for 1 minute. The reaction was recorded similarly to CMT based on gel formation (Muhammad et al., 2010).

2.3.2 Modified Surf Field Mastitis Test (MSFMT)

The MSFMT was developed by modification of SFMT reagent through heating to 50°C and adjusting pH to 8-9 using hydrochloric acid (Muhammad et al., 1995). The test procedure remained same to SFMT, but with enhanced clarity of results.

2.4 Somatic Cell Count Analysis

2.4.1 Stain Preparation

Newman's Lampert stain was developed according to Schalm et al. (1971) with modifications by Doxey (1971). The stain contained 1.12 gm methylene blue dissolved in 54 ml 95 % ethanol, mixed with 40ml tetrachloroethane, heated at 55-60°C for 30 minutes, following cooled before adding 6ml glacial acetic acid.

2.4.2 Slide Preparation and Counting

Milk smears (10 µl) were prepared on clean slides and air-dried, following defatted in xylene for 5-6 minutes (Ahmad et al., 2009). Following staining, slides were examined under oil immersion (100X) and somatic cells were counted in 100 fields. The Somatic Cell Count was calculated using the formula: SCC (Cells/ml) = Average Cells per field × 40,000 (Schalm et al., 1971).

2.5 Statistical Analysis

Test findings were compared using correlation analysis. A threshold of >150,000 cells/ml was used to define sub-clinical mastitis based on Swedish Dairy Association standards. The agreement between different test methods was assessed using kappa statistics (Muhammad et al., 2010).

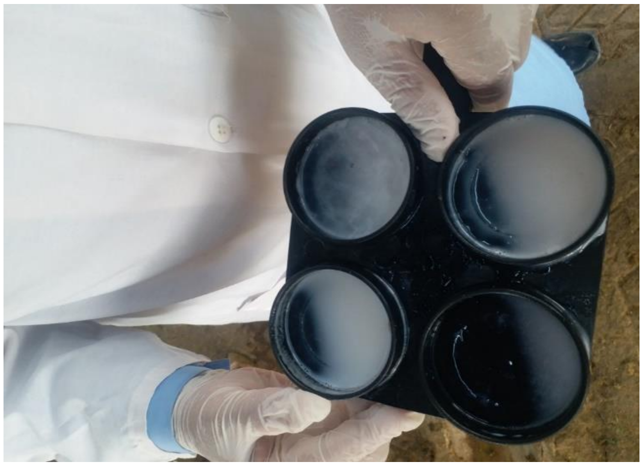


Figure 2.1 Interpretation of Surf Field Mastitis Test

Figure 2.2 Interpretation of California Mastitis Test

Fig 2.3 Collection of Milk

Fig 2.4 Preparation of slides for SSC

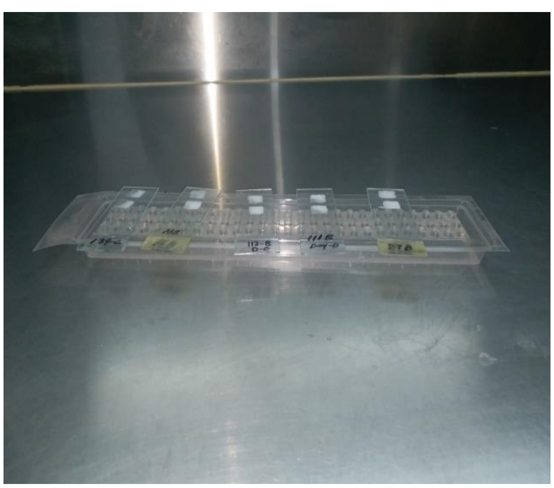


Fig 2.5 Milk Slides staining for Newman's Lambert stain

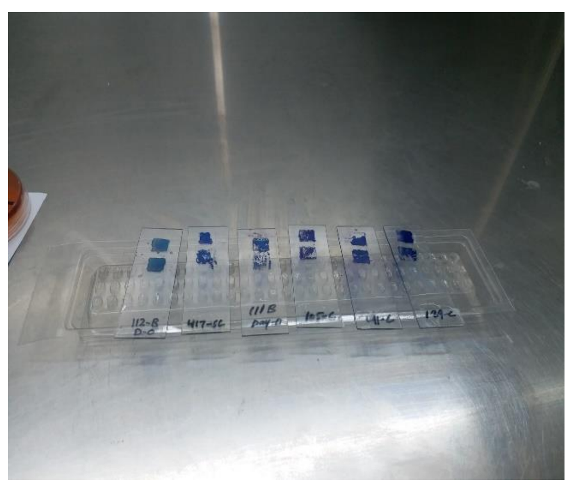


Fig 2.6 Stained Slides after staining with Newman's lambert stain

Figure 2.7 Counting of somatic cell in microscope

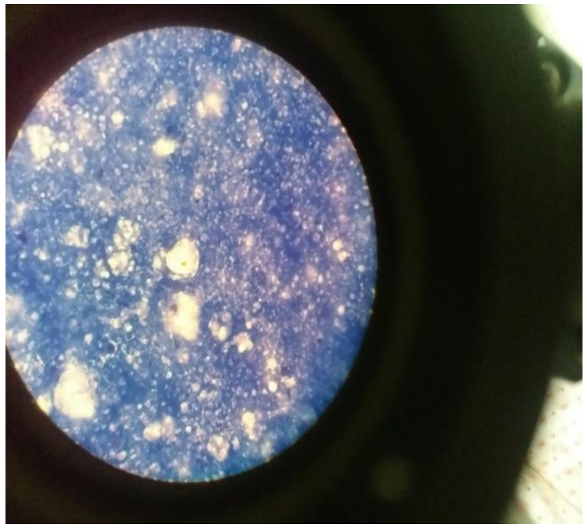


Figure 3.8 microscopic views of somatic cells in a stained glass slide

Results

3.1 Frequency Distribution of Mastitis Test Results in Cows

The Modified Surf Field Mastitis Test (MSFMT) detected sub-clinical mastitis in 55.5 % of cattle teats (111/200), with severity grades distributed as +1 (30.5%), +2 (20.0%), and +3 (5.0%). The California Mastitis Test (CMT) exhibited higher sensitivity, identifying 67.18 % positive cases (129/192), including +1 (29.5%), +2 (23.0%), and +3 (12.0%) reactions. The Surf Field Mastitis Test (SFMT) shown the lowest detection rate at 47.91 % (92/192), with +1 (26.0%), +2 (14.5%), and +3 (5.5%) classifications.

3.2 Frequency Distribution of Mastitis Test Results in Buffaloes

MSFMT in buffaloes, detected 48.2 % positive cases (96/199), with +1 (32.5%), +2 (13.0%), and +3 (2.5%) grades. CMT again demonstrated superior sensitivity, identifying 59.2% positives (119/199), including +1 (34.0%), +2 (17.5%), and +3 (8.0%) reactions. SFMT identified 44.2% positives (88/199), with +1 (33.0%), +2 (9.5%), and +3 (1.5%) classifications.

Table 3.1: Frequency distribution of cows with respect to Modified Surf Field Mastitis Test

MSFMT	**Frequency**	**Percent**	**Percent (out of 192)**
-ve	81	40.5	42.2
+1	61	30.5	31.8
+2	40	20.0	20.8
+3	10	5.0	5.2
Total	**192**	**96.0**	**100.0**
Bl	**8**	**4.0**	
Overall	**200**	**100.0**	

Bl= blind teats with no milk, **+1**= visible light gel by transparence, **+2**= visible gel adhesion to cup, viscous filament, **+3**= strong gel like the white of egg.

Table 3.2: Frequency distribution of cows with respect to California Mastitis Test (CMT)

CMT	Frequency	Percent	Percent (out of 192)
-ve	63	31.5	32.8
+1	59	29.5	30.7
+2	46	23.0	24.0
+3	24	12.0	12.5
Total	**192**	**96.0**	**100.0**
Bl	**8**	**4.0**	
Overall	**200**	**100.0**	

Bl= blind teats with no milk, **+1**= visible light gel by transparence, **+2**= visible gel adhesion to cup, viscous filament, **+3**= strong gel like the white of egg.

Table 3.3: Frequency distribution of cows with respect to Surf Field Mastitis Test (SFMT)

SFMT	Frequency	Percent	Percent (out of 192)
-ve	100	50.0	52.1
+1	52	26.0	27.1
+2	29	14.5	15.1
+3	11	5.5	5.7
Total	**192**	**96.0**	**100.0**
Bl	**8**	**4.0**	
Overall	**200**	**100.0**	

Bl= blind teats with no milk, **+1**= visible light gel by transparence, **+2**= visible gel adhesion to cup, viscous filament, **+3**= strong gel like the white of egg.

Table 3.4: Frequency distribution of buffaloes with respect to Modified Surf Field Mastitis Test (MSFMT)

MSFMT	Frequency	Percent	Percent (out of 199)
-ve	103	51.5	51.8
+1	65	32.5	32.7
+2	26	13.0	13.1
+3	5	2.5	2.5
Total	**199**	**99.5**	**100.0**
Bl	**1**	**0.5**	
Overall	**200**	**100.0**	

Bl= blind teats with no milk, **+1**= visible light gel by transparence, **+2**= visible gel adhesion to cup, viscous filament, **+3**= strong gel like the white of egg.

Table 3.5: Frequency distribution of buffaloes with respect to California Mastitis Test (CMT)

CMT	Frequency	Percent	Percent (out of 199)
-ve	80	40.0	40.2
+1	68	34.0	34.2
+2	35	17.5	17.6
+3	16	8.0	8.0
Total	**199**	**99.5**	**100.0**
Bl	**1**	**0.5**	
Overall	**200**	**100.0**	

Bl= blind teats with no milk, **+1**= visible light gel by transparence, **+2**= visible gel adhesion to cup, viscous filament, **+3**= strong gel like the white of egg.

Table 3.6: Frequency distribution of buffaloes with respect to Surf Field Mastitis Test (SFMT)

SFMT	Frequency	Percent	Percent (out of 199)

-ve	111	55.5	55.8
+1	66	33.0	33.2
+2	19	9.5	9.5
+3	3	1.5	1.5
Total	**199**	**99.5**	**100.0**
Bl	**1**	**0.5**	
Overall	**200**	**100.0**	

Bl= blind teats with no milk, **+1=** visible light gel by transparence, **+2=** visible gel adhesion to cup, viscous filament, **+3=** strong gel like the white of egg.

3.3 Somatic Cell Count (SCC) Analysis in Cows

SCC elevated significantly (P < 0.01) with mastitis severity across all tests. For MSFMT, SCC increased from 253,756 cells/mL (negative) to 939,745 cells/mL (+3). CMT demonstrated a similar trend, with SCC enhancing from 229,906 cells/mL (negative) to 762,315 cells/mL (+3). SFMT results observed with the same pattern, with SCC ranging from 267,764 cells/mL (negative) to 819,587 cells/mL (+3). Highly significant differences (P < 0.01) between severity grades for all tests were confirmed through ANOVA.

3.4 Somatic Cell Count (SSC) Analysis in Buffaloes

Buffaloes revealed higher baseline SCC than cows. MSFMT results exhibited SCC increasing from 338,675 cells/mL (negative) to 1,244,432 cells/mL (+3). CMT results ranged from 315,704 cells/mL (negative) to 1,061,259 cells/mL (+3), while SFMT results observed with 368,162 cells/mL (negative) to 1,040,927 cells/mL (+3). All tests revealed highly significant (P < 0.01) SCC differences across severity grades.

Table 3.7: Mean value of somatic cell count per ml in cows with respect to Modified Surf Field Mastitis Test

MSFMT	N	Mean	SD	SE	95% CI for Mean		Min	Max
					LB	UB		
-ve	81	253756	73847	8205	237427	270085	180801	560393
+1	61	327737	111000	14212	299309	356166	201149	770163
+2	40	565741	237982	37628	489631	641852	280223	1659509
+3	10	939745	248421	78558	762035	1117454	561000	1242303
Total	192	377986	227778	16438	345562	410410	180801	1659509

SD = Standard deviation; SE = Standard error; CI = Confidence interval; LB = Lower bound, UB = Upper bound; Min = Minimum value; Max = Maximum value

Table 3.8 Analysis of variance (ANVOA) table for SCC/ml in cows regarding MSFMT

Source of variation	Sum of Squares	df	Mean Square	F-value	P-value
Between Groups	5.96991E+12	3	1.98997E+12	94.96	0.0000
Within Groups	3.93973E+12	188	20956007719		
Total	9.90964E+12	191			

** = Highly significant (P<0.01)

3.5 Quarter – Wise SCC Distribution in Cows

No significant positional differences (P > 0.05) were experienced in SCC distribution across udder quarters. Mean SCC values for right front, right rear, left front, and left rear quarters were 420,691 ±

233,424, 365,631±244,710, 389,845±264,535, and 335,915±148,380, cells/mL, respectively. Highly significant ($P < 0.01$) associations were confirmed between test results and SCC through Statistical analysis.

3.6 Quarter-Wise SCC Distribution in Buffaloes

Similar to cows, buffaloes exhibited no significant quarter-wise SCC variation ($P > 0.05$). Mean SCC values for right front, right rear, left front, and left rear quarters were 504,457 ± 291,047, 485,679 ± 268,102, 495,329 ± 221,964, and 550,679 ± 321,282 (cells/mL) respectively. All tests retained strong correlations ($P < 0.01$) with SCC.

Means Plot

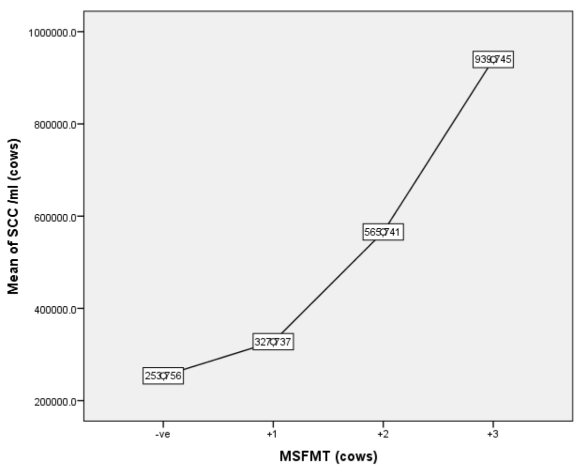


Figure 3.1 Graph showing SCC/ml in cows wrt MSFMT

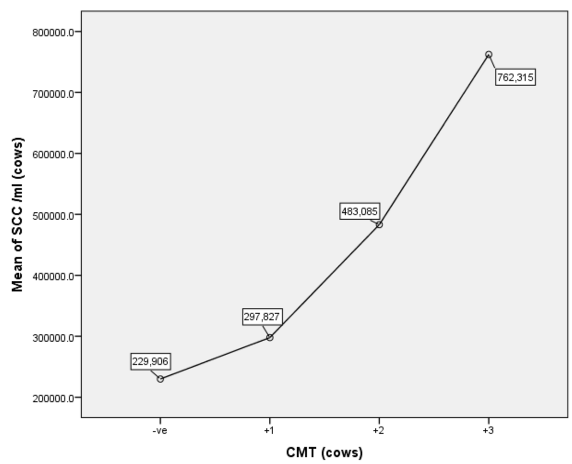


Figure 3.2 Graph showing SCC/ml in cows wrt CMT

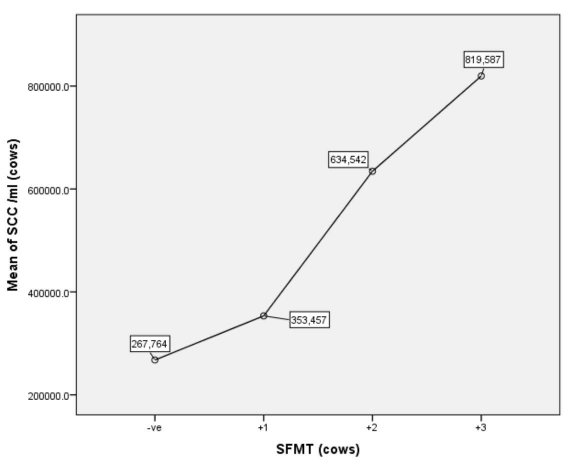


Figure 3.3 Graph showing SCC/ml in cows wrt SFMT

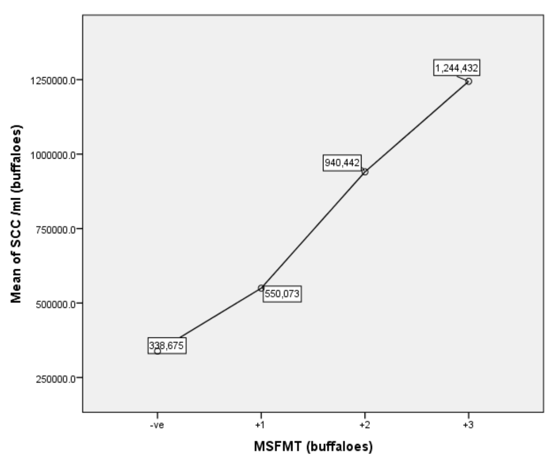


Figure 3.4 Graph showing SCC/ml in buffaloes wrt MSFMT

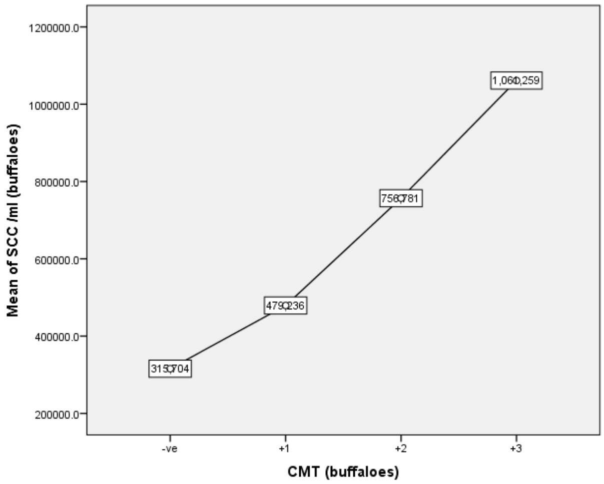


Figure 3.5 Graph showing SCC/ml in buffaloes wrt CMT

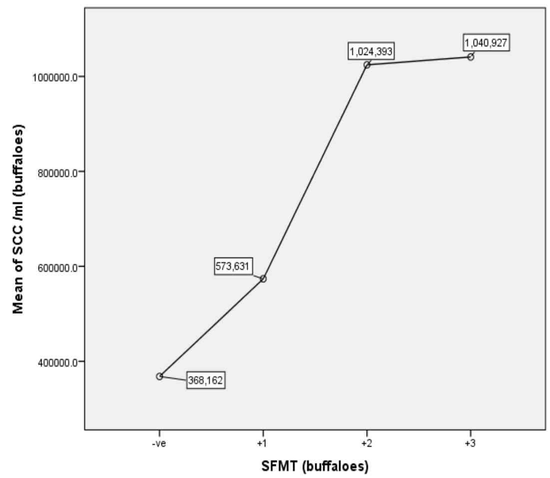


Figure 3.6 Graph showing SCC/ml in buffaloes wrt SFMT

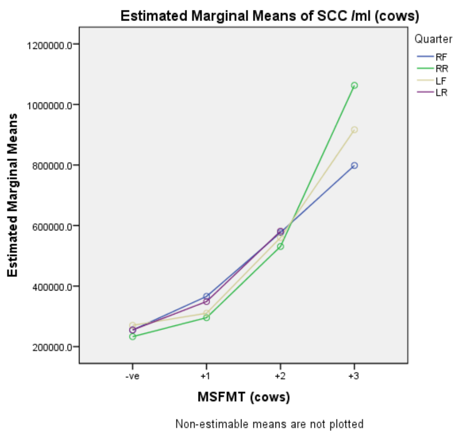


Figure 3.7 Graph showing quarter wise relation SCC/ml in cows wrt MSFMT

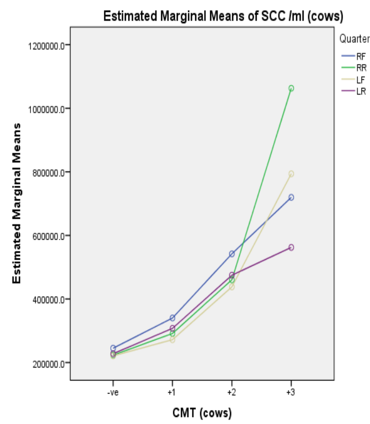


Figure 3.8 Graph showing quarter wise relation SCC/ml in cows wrt CMT

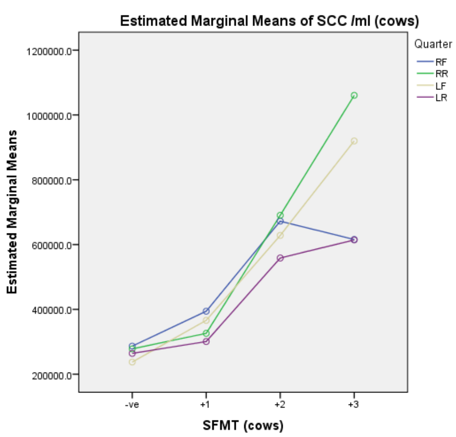


Figure 3.9 Graph showing quarter wise relation SCC/ml in cows wrt SFMT

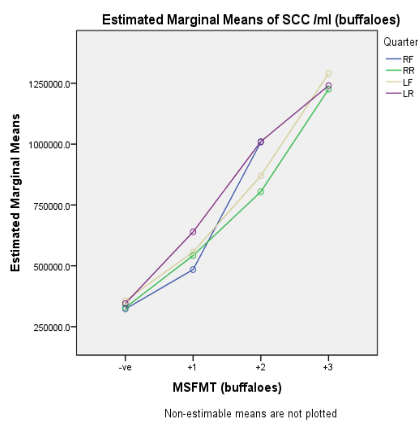
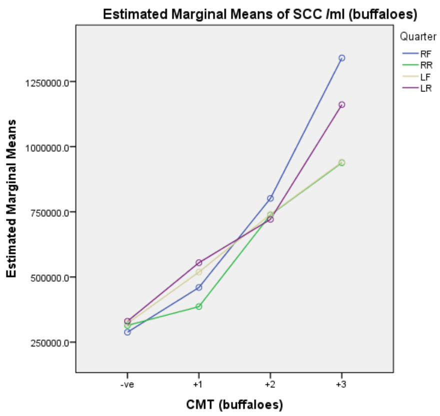


Figure 3.10 Graph showing quarter wise relation SCC/ml in Buffaloes wrt MSFMT



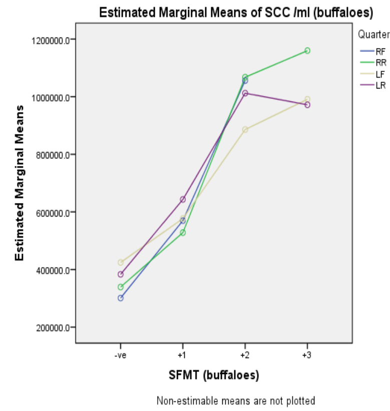
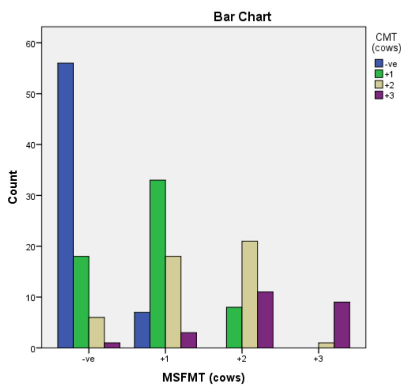


Figure 3.11 Graph showing quarter wise relation SCC/ml in Buffaloes wrt CMT

Figure 3.12 Graph showing quarter wise relation SCC/ml in Buffaloes wrt SFMT



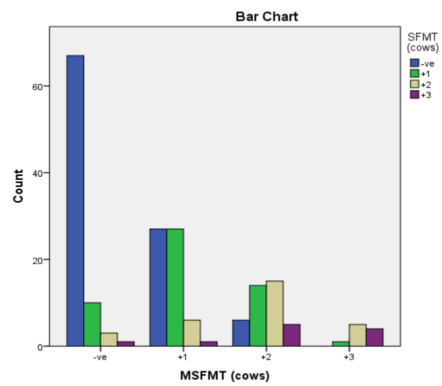
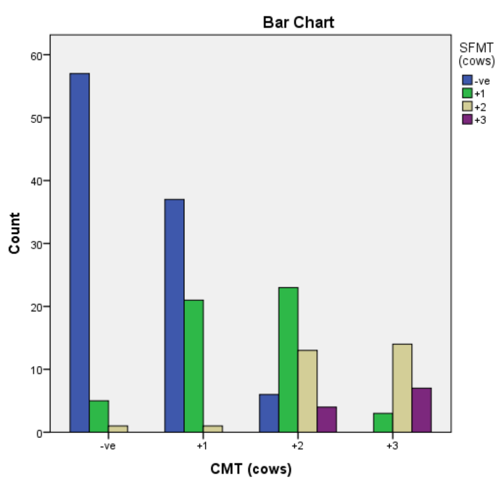


Figure 3.13 Graph showing comparative relation between MSFMT and CMT

Figure 3.14 Graph showing comparative relation between MSFMT and SFMT



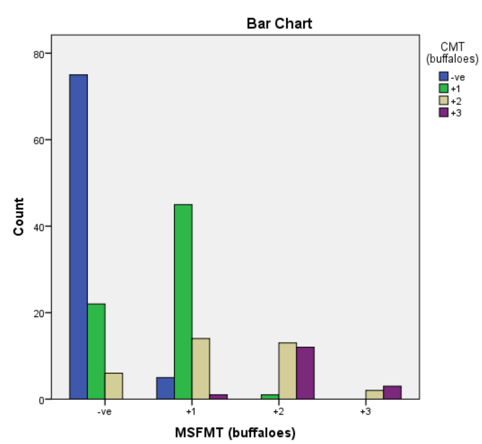


Figure 3.15 Graph showing comparative relation between SFMT and CMT

Figure 3.16 Graph showing comparative relation between MSFMT and CMT

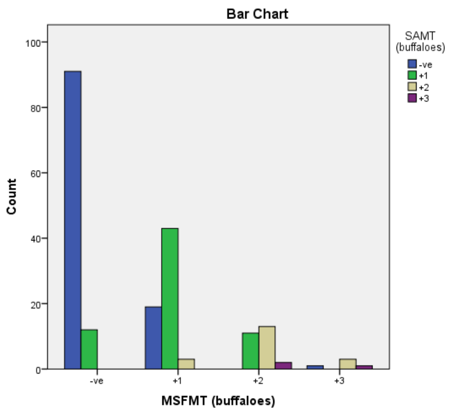


Figure 3.17 Graph showing comparative relation between MSFMT and SFMT

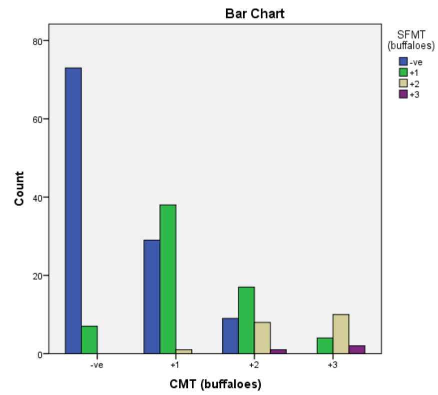


Figure 3.18 Graph showing comparative relation between SFMT and SFMT

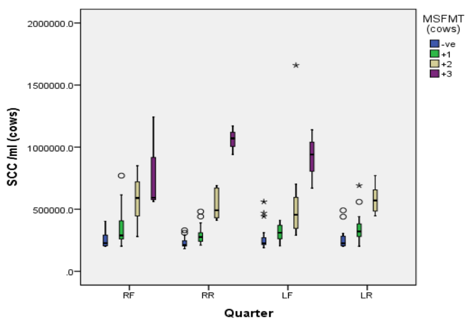


Figure 3.19 showing elevated somatic cells level of individual quarter w.r.t MSMFT in cows

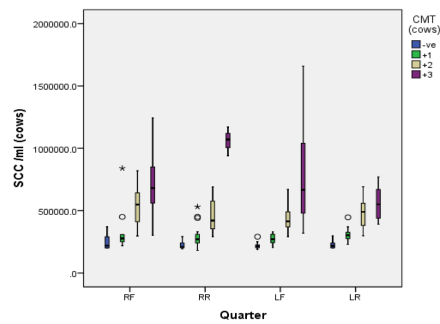


Figure 3.20 showing elevated somatic cells level of individual quarter w.r.t CMT in cows

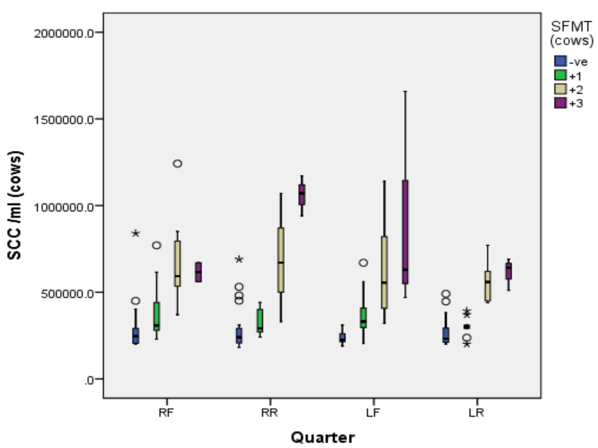


Figure 3.21 showing elevated somatic cells level of individual quarter w.r.t SMFT in cows

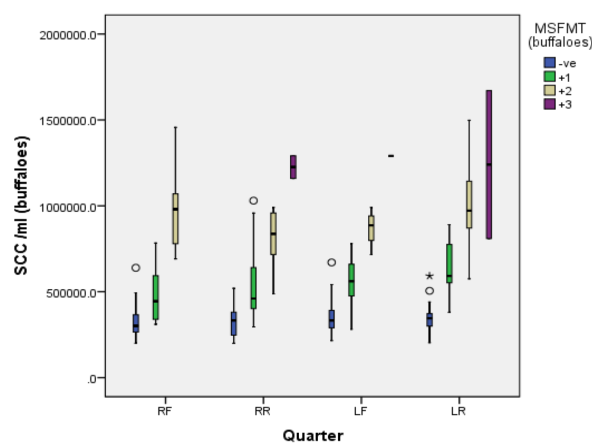


Figure 3.22 showing elevated somatic cells level of individual quarter w.r.t MSMFT in Buffaloes

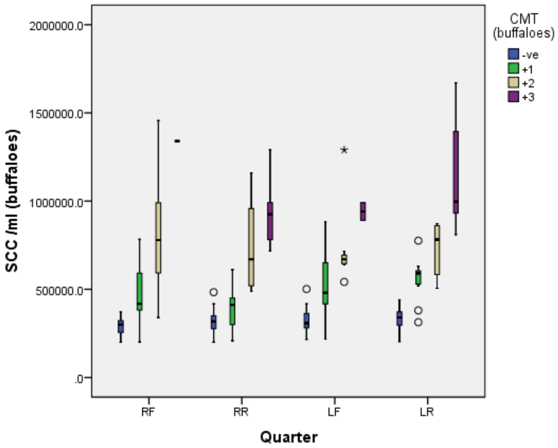


Figure 3.23 showing elevated somatic cells level of individual quarter w.r.t MSMFT in Buffaloes

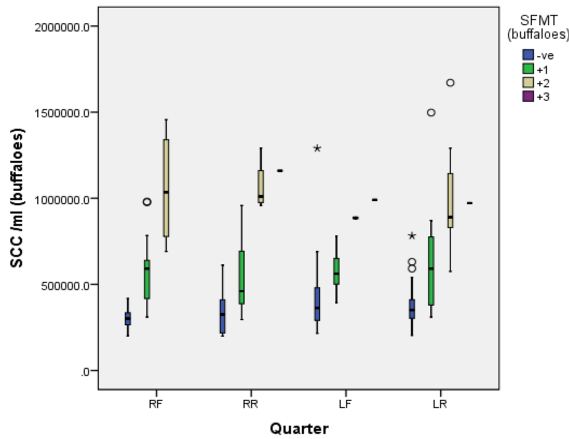


Figure 3.24 showing elevated somatic cells level of individual quarter w.r.t SMFT in Buffaloes

3.7 Comparative Efficacy of Mastitis in Cows

CMT revealed the highest diagnostic accuracy, detecting 12.5% +3 cases compared to MSFMT (5.2%) and SFMT (5.7%). Statistical analysis (Chi-square = 104.00–165.50, $P < 0.01$; Gamma = 0.769–0.881) observed CMT's superior performance.

3.8 Comparative Efficacy of Mastitis Tests in Buffaloes

CMT again outperformed MSFMT and SFMT, detecting 8.0% +3 cases versus 2.5% (MSFMT) and 1.5% (SFMT). Statistical comparisons (Chi-square = 143.78–194.94, $P < 0.01$; Gamma = 0.850–0.922) confirmed CMT's higher efficacy.

3.9 Test Agreement and Correlation Analysis

A high significance ($P < 0.01$) were observed amongst all inter-test correlations. CMT and MSFMT exhibited the strongest agreement (Gamma = 0.854 in cows, 0.907 in buffaloes), while SFMT demonstrated weaker correlations. ANOVA models explained ~90% of SCC variance ($R^2 = 0.900$), supporting the robustness of the findings.

3.10 Overall Diagnosis Performance

The hierarchical efficacy pattern was observed across species as CMT > MSFMT > SFMT. CMT's higher sensitivity for severe cases (+3) and stronger SCC correlations claimed it as the most reliable field test. MSFMT, while improved over SFMT, remained less accurate as compare to CMT.

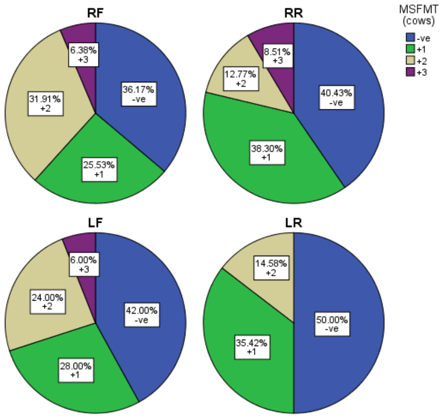


Figure 3.25 showing quarter wise distribution of MSMFT in cows

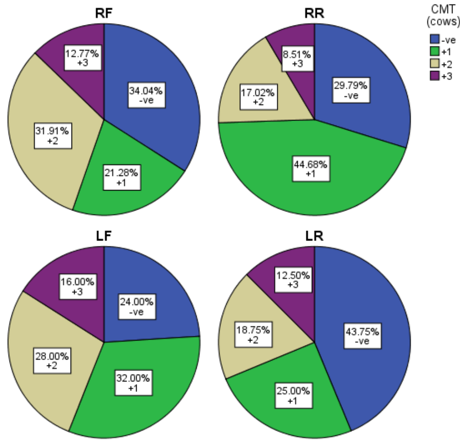


Figure 3.26 showing quarter wise distribution of CMT in cows

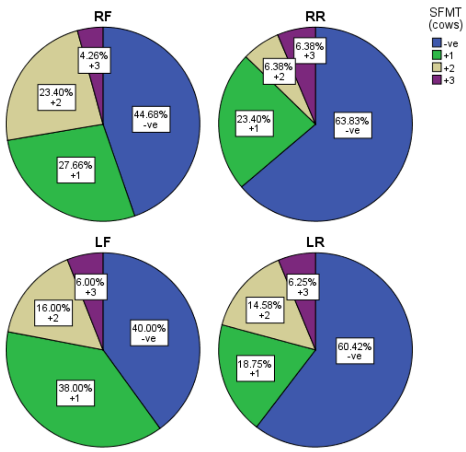


Figure 3.27 showing quarter wise distribution of SMFT in cows

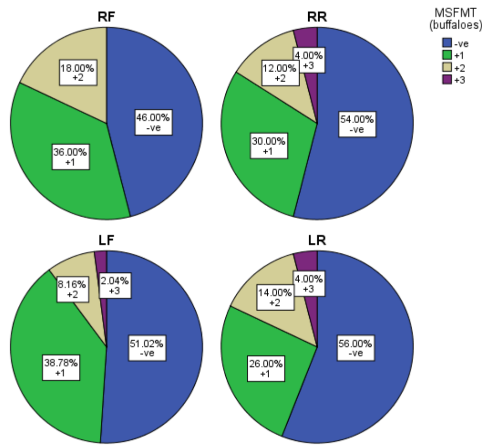


Figure 3.28 showing quarter wise distribution of SMFT in Buffaloes

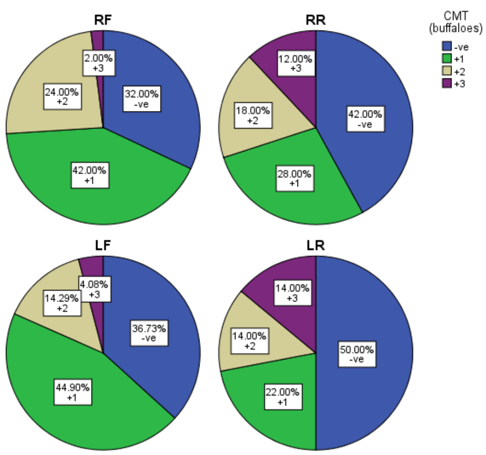


Figure 3.29 showing quarter wise distribution of CMT in Buffaloes

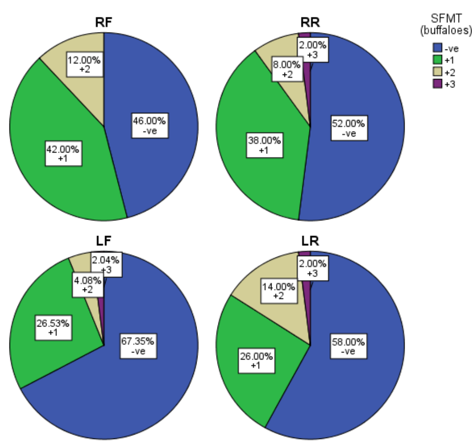


Figure 3.30 showing quarter wise distribution of SFMT in Buffaloes

Discussion

Mastitis remains a major and crucial constraint in dairy production, causing substantial economic losses through reduced milk yield, increased culling rates, and treatment costs (Halasa et al., 2007). Our research study compared three diagnostic tests (CMT, MSFMT, and SFMT) for subclinical mastitis detection in cattle and buffaloes, exhibiting significant differences in their efficacy.

The CMT revealed the highest sensitivity, detecting 67.18% positive cases in cows and 59.2% in buffaloes, consistent with Sharma et al. (2010) who reported CMT's superior accuracy (72.4%) compared to other field tests. However, our detection rates of sub-clinical mastitis were higher than Badiuzzaman et al. (2015) (52.25% +1 cases), possibly due to differences in herd management or regional pathogen prevalence patterns (Kivaria et al., 2007). The MSFMT revealed improved performance over conventional SFMT (55.5% vs 47.91% detection in cows), supporting Muhammad et al. (2010) results that modified detergent-based tests enhance diagnostic sensitivity.

Somatic cell counts (SCC) exhibited a strong positive correlation with test positivity grades ($P<0.01$), validating their use as mastitis indicators (Dohoo and Leslie, 1991). The SCC elevation from 253,756 cells/mL (negative) to 939,745 cells/mL (+3) in cows aligns with international thresholds where >200,000 cells/mL indicates infection (Idriss et al., 2013). Buffaloes showed higher baseline SCCs (338,675 cells/mL), corroborating Tripaldi et al. (2010) who reported naturally elevated SCC in buffalo milk.

Quarter-wise investigation shown no positional SCC differences ($P>0.05$), contradicting some studies reporting higher infection rates in rear quarters (Sargeant et al., 2001). This consistency suggests systemic rather than localized inflammatory responses in our herds, possibly due to improved milking hygiene practices (Barkema et al., 1999).

Economically, our results support early detection, as subclinical mastitis causes 40% greater production losses than clinical cases (Seegers et al., 2003). The high +1/+2 case prevalence (53.5% in cows) emphasizes the need for regular screening advocated by Ruegg (2017). While CMT remains the gold standard (Radostits et al., 2007), MSFMT's cost-effectiveness makes it practical for small holder farms (Mdegela et al., 2004).

These findings emphasize the importance of context-specific mastitis control programs. Future studies should validate these tests against bacteriological culture across different management systems, as recommended by Pyörälä (2009), while exploring affordable alternatives for resource-limited settings.

Conclusions

1. CMT revealed significantly higher diagnostic accuracy than MSFMT and SFMT for subclinical mastitis detection in both cattle and buffaloes.
2. All tests exhibited strong correlations with somatic cell counts, validating their use as field diagnostic tools.
3. The modified MSFMT demonstrated improved sensitivity over conventional SFMT, suggesting its potential as an affordable alternative.
4. Uniform SCC distribution across udder quarters shows systemic rather than localized infection patterns in the studied herds.

Recommendations

1. Dairy farms should adopt CMT as the primary screening tool for sub-clinical mastitis detection.
2. MSFMT can be implemented in resource-limited settings where CMT reagents are unavailable.
3. Monthly regular testing should be incorporated into herd health programs, particularly during early lactation.

4. Future studies should evaluate these tests bacteriological correlation across different production systems.

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