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Evaluating Diagnostic Tests for Bovine Mastitis: A Comparative Analysis of CMT, SCC, and EC

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Abstract: Bovine mastitis, an inflammation of the mammary gland, poses a significant economic burden on the dairy industry globally. Timely and accurate diagnosis is crucial for effective mastitis management. This paper critically analyzes the performance of three commonly used diagnostic tests for bovine mastitis: the California Mastitis Test (CMT), Somatic Cell Count (SCC), and Electrical Conductivity (EC). Based on presented data, we discuss the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each test, considering their ability to detect both Gram-positive and Gram-negative bacteria. This evaluation highlights the strengths and limitations of each test, emphasizing the need for a combined approach integrating rapid on-farm tests with bacteriological culture for optimized mastitis detection and management strategies.

Keywords: Bovine Mastitis, California Mastitis Test, Somatic Cell Count, Electrical Conductivity, Diagnostic Accuracy, Gram-positive bacteria, Gram-negative bacteria.

INTRODUCTION

Bovine mastitis remains a prevalent and costly disease in dairy farming, impacting milk production, quality, and animal welfare (Halasa, *et al*., 2009). Economic losses associated with mastitis stem from reduced milk yield, discarded milk due to antibiotic treatment, veterinary expenses, and premature culling of affected cows (Ruegg, 2017). Effective mastitis control relies heavily on prompt and accurate diagnosis. Traditional diagnostic methods, such as bacteriological culture of milk samples, are considered the gold standard for identifying specific pathogens and guiding targeted antibiotic therapy (Watts, 1997). However, culture is time-consuming, requiring 24-48 hours for results, and expensive, making it impractical for routine screening and immediate treatment decisions. Furthermore, the sensitivity of culture can be affected by intermittent shedding of pathogens and prior antibiotic treatment (Keefe, 2012). Consequently, rapid on-farm tests like the California Mastitis Test (CMT), Somatic Cell Count (SCC), and Electrical Conductivity (EC) have gained widespread adoption as valuable screening tools for early mastitis detection.

The CMT is a subjective assessment that detects elevated levels of somatic cells by reacting with DNA and causing gel formation (Schalm & Noorlander, 1957). SCC provides an objective measurement of the number of somatic cells (primarily leukocytes) in milk, reflecting the inflammatory response to infection (Harmon, 1994). EC measures the electrical resistance of milk, which is altered by changes in ion concentrations associated with mammary gland inflammation (Linzell & Peaker, 1972).

This paper evaluates the performance of CMT, SCC, and EC in detecting mastitis-causing pathogens, specifically focusing on their ability to differentiate, albeit indirectly, between Gram-positive and Gram-negative bacteria. By analyzing the sensitivity, specificity, PPV, and NPV of each test, we provide insights into their diagnostic accuracy and guide the development of more effective mastitis management strategies that balance the need for rapid on-farm decisions with the accuracy of identifying specific pathogens.

MATERIALS AND METHODS

This analysis utilizes data providing detection rates, sensitivity, specificity, PPV, and NPV of CMT, SCC, and EC for detecting Gram-positive and Gram-negative bacteria. This data is based on laboratory work conducted at Adami Tulu Agricultural Research Center, one of the centers of Oromia Agricultural Research Institute, specifically in the Veterinary Microbiology Laboratory.

Milk samples were collected from 200 cows with subclinical mastitis, all of which were in their 2nd or 3rd parity, from surrounding areas of Meki and Batu towns. The reference standard for identifying mastitis-causing pathogens was bacteriological culture, conducted following standard procedures, including the National Mastitis Council (NMC) guidelines. Gram-positive and Gram-negative bacterial infections of the mammary gland were confirmed through bacteriological culture, which

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served as the gold standard against which the other test results were validated.

The following definitions are relevant for evaluating the performance of diagnostic tests:

- **Sensitivity:** Ability of the test to correctly identify cows with mastitis (**true positive rate**).
  - **Formula:** True Positives / (True Positives + False Negatives)
- **Specificity:** Ability of the test to correctly identify cows without mastitis (**true negative rate**).
  - **Formula:** True Negatives / (True Negatives + False Positives)
- **Positive Predictive Value (PPV):** Probability that a cow with a positive test result truly has mastitis.
  - **Formula:** True Positives / (True Positives + False Positives)
- **Negative Predictive Value (NPV):** Probability that a cow with a negative test result truly does not have mastitis.
  - **Formula:** True Negatives / (True Negatives + False Negatives)

RESULTS AND DISCUSSION

Detection Rates

The detection rates for CMT, SCC, and EC are presented in Table 1. All tests showed reasonable detection rates, with CMT having the highest overall detection rate (72.5%) and SCC having the lowest (65.2%). However, when examining Gram-positive and Gram-negative bacteria separately, CMT showed a higher detection rate for Gram-positive bacteria (76.1%) compared to Gram-negative bacteria (62.7%). SCC exhibited a similar trend, with a higher detection rate for Gram-positive bacteria (68.4%) than for Gram-negative (55.6%). EC had a higher detection rate for Gram-negative bacteria (72.1%) compared to Gram-positive bacteria (60.9%).

These detection rates highlight the varying sensitivities of each test for different types of bacteria. Gram-positive bacteria, such as *Staphylococcus aureus* and *Streptococcus agalactiae*, are often associated with chronic mastitis, while Gram-negative bacteria, such as *Escherichia coli* and *Klebsiella pneumoniae*, are more commonly associated with acute, severe mastitis (Bradley, 2002). The differences in detection rates suggest that each test may be more appropriate for detecting certain types of infections.

Table 1: Detection Rates

Test Type	Overall	Gram-positive	Gram-negative
CMT	72.5%	76.1%	62.7%
SCC	65.2%	68.4%	55.6%
EC	68.8%	60.9%	72.1%

Sensitivity and Specificity

CMT exhibited the highest sensitivity for Gram-positive bacteria (77.4%) but lower sensitivity for Gram-negative bacteria (59.7%). SCC had a slightly lower sensitivity for Gram-positive bacteria (74.3%) and a considerably lower sensitivity for Gram-negative bacteria (48.9%). EC showed the lowest sensitivity for Gram-positive bacteria (58.6%) and moderate sensitivity for Gram-negative bacteria (68.9%).

The specificity values indicate the ability of each test to correctly identify cows without mastitis. EC demonstrated the highest specificity for both Gram-positive and Gram-negative bacteria (86.7% and 82.4%, respectively), suggesting a lower risk of false positive results. CMT had moderate specificity (78.3% for Gram-positive and 76.2% for Gram-negative), while SCC had the lowest specificity for Gram-positive bacteria (70.6%).

These results suggest that CMT may be a useful screening tool for detecting Gram-positive mastitis due to its high sensitivity. However, its lower specificity could lead to unnecessary treatment of uninfected cows. SCC appears to be less reliable for detecting Gram-negative mastitis due to its lower sensitivity. EC, with its higher specificity, may be useful for ruling out mastitis, particularly Gram-positive infections.

Table 2: Sensitivity and Specificity

Test Type	Gram-positive Sensitivity	Gram-negative Sensitivity	Gram-positive Specificity	Gram-negative Specificity
CMT	77.4%	59.7%	78.3%	76.2%
SCC	74.3%	48.9%	70.6%	80.1%
EC	58.6%	68.9%	86.7%	82.4%

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3.3 Positive and Negative Predictive Values

The PPV and NPV provide further insight into the clinical utility of each test. The PPV indicates the probability that a cow with a positive test result truly has mastitis, while the NPV indicates the probability that a cow with a negative test result truly does not have mastitis.

SCC has the lowest PPV for both Gram-positive and Gram-negative bacteria, indicating that a positive SCC result is more likely to be a false positive, leading to potential overtreatment and unnecessary antibiotic use. EC demonstrates the highest PPV for Gram-negative bacteria (72.3%), suggesting that a positive EC result is more likely to indicate a true Gram-negative infection. The relatively high NPV values for all tests suggest that a negative result is a good indicator that the cow is not infected.

Table 3: PPV and NPV

Test Type	Gram-positive PPV	Gram-negative PPV	Gram-positive NPV	Gram-negative NPV
CMT	65.8%	58.1%	86.2%	74.5%
SCC	56.6%	41.8%	85.7%	69.3%
EC	63.5%	72.3%	81.4%	79.6%

INTERPRETATION AND IMPLICATIONS

Based on the data presented, the following interpretations and implications can be drawn:

- **CMT:** Highly sensitive for Gram-positive bacteria but lower specificity, leading to a higher risk of false positives. This test might be best used as an initial screening tool, with positive results followed by more specific tests.
- **SCC:** Has a lower PPV, indicating a higher likelihood of false positives and potential overtreatment. While SCC is valuable for monitoring overall herd health, it may not be the most reliable indicator of individual cow infections, especially Gram-negative.
- **EC:** More specific for Gram-negative bacteria, suggesting its utility as a complementary test to SCC and CMT. A positive EC result could warrant further investigation for Gram-negative infections.

Therefore, relying solely on one test can lead to suboptimal management decisions. For example, using only SCC as a diagnostic tool might lead to unnecessary antibiotic use due to false positives. A more effective approach involves integrating these tests into a comprehensive mastitis management program. For instance, a farmer could use CMT for initial screening, followed by SCC to quantify the level of inflammation, and then EC to potentially differentiate between Gram-positive and Gram-negative infections. Cows with suspicious results should then undergo bacteriological culture for definitive diagnosis and targeted treatment.

LIMITATIONS

This analysis is based on the presented data without details on test procedures, bacterial culture protocols, and environmental factors, which may influence results. The absence of these details represents a significant limitation. Factors such as the experience of the person performing the CMT, the specific SCC measurement method, the cut-off values used to define a positive result, and the specific bacteriological culture protocols employed can all impact the accuracy and reliability of the results (Dohoo, *et al*., 2011). Additionally, the prevalence of different mastitis-causing pathogens in the herd can influence the PPV and NPV of the tests (Thrusfield, 2007). Moreover, the data does not account for variations in host immune responses, which can affect the relationship between test results and bacterial presence. Further research is needed to validate these findings in diverse dairy farming settings.

CONCLUSION

This comparative analysis highlights the varying performance characteristics of CMT, SCC, and EC in detecting bovine mastitis. While CMT showed a higher overall detection rate, SCC demonstrated low PPV, suggesting a higher risk of false positives. EC exhibited higher specificity and PPV for Gram-negative bacteria.

RECOMMENDATIONS:

- **Utilize a combination of tests:** Implementing a screening protocol that integrates CMT, SCC, and EC can improve diagnostic accuracy and allow for a more nuanced understanding of the infection.
- **Consider Gram-stain results (where available):** Preliminary milk samples analysis via gram stain would allow for rapid assessment of likely causative organism to guide treatment decisions.
- **Regular bacteriological culture:** Periodic culture of milk samples from cows with elevated SCC or positive CMT/EC results is crucial for identifying specific pathogens and

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guiding targeted antibiotic therapy, minimizing unnecessary antibiotic use and promoting responsible antimicrobial stewardship.

- **Establish farm-specific cut-off values:** Determine the optimal cut-off values for SCC and EC based on the specific herd's health status and management practices.
- **Implement comprehensive mastitis control programs:** Focus on preventive measures such as proper milking hygiene, dry cow therapy, and environmental management to minimize the incidence of mastitis.

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