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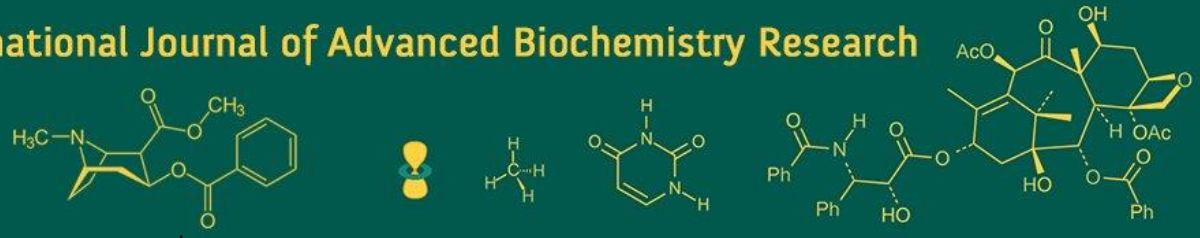

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Mansoor Nabi
Bacteriology Laboratory, Division of Veterinary Microbiology, FVSC&A.H Shuhama (Alusteng) SKUAST-K, Jammu & Kashmir, India

Md. Isfaqul Hussain
Bacteriology Laboratory, Division of Veterinary Microbiology, FVSC&A.H Shuhama (Alusteng) SKUAST-K, Jammu & Kashmir, India

Sabia Qureshi
Bacteriology Laboratory, Division of Veterinary Microbiology, FVSC&A.H Shuhama (Alusteng) SKUAST-K, Jammu & Kashmir, India

Zahid Amin Kashoo
Bacteriology Laboratory, Division of Veterinary Microbiology, FVSC&A.H Shuhama (Alusteng) SKUAST-K, Jammu & Kashmir, India

Nahida Nabi
Department of Education, Central University of Kashmir, Jammu & Kashmir, India

Corresponding Author:
Mansoor Nabi
Bacteriology Laboratory, Division of Veterinary Microbiology, FVSC&A.H Shuhama (Alusteng) SKUAST-K, Jammu & Kashmir, India

Prevalence of clinical and subclinical bovine mastitis in Kashmir, India: Evaluating diagnostic methods and implications for udder health

Mansoor Nabi, Md. Isfaqul Hussain, Sabia Qureshi, Zahid Amin Kashoo and Nahida Nabi

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Abstract
This study investigated the prevalence of clinical and subclinical bovine mastitis in Kashmir, India, and evaluated four diagnostic methods: California Mastitis Test (CMT), Infrared Thermography, Bacterial Culture, and Somatic Cell Count (SCC). Analysing 394 composite milk samples and infrared thermal images from crossbred dairy cattle, we found that 9.4% (37/394) had clinical mastitis, while subclinical mastitis affected 26.4% (104/394) based on SCC thresholds. The Somatic Cell Count (SCC) method identified a total of 141 cases. In comparison, Infrared Thermography detected 118 cases (83.69%), Bacterial Culture identified 96 cases (68.09%), and the California Mastitis Test (CMT) detected 85 cases (60.28%) of the total cases identified by the SCC method. Overall, mastitis was present in over one-third of the sampled population, underscoring a significant animal health concern. These findings highlight the necessity for comprehensive mastitis control programs in Kashmir, particularly for subclinical cases, to enhance udder health and sustain the dairy industry through regular screening and early intervention.

Keywords: CMT, infrared, somatic cell count, prevalence, mastitis

Introduction

Bovine mastitis is a common and costly disease affecting dairy cattle worldwide. In Kashmir, where dairy farming is a vital part of the agricultural economy, understanding the prevalence of mastitis is crucial for developing effective control measures. Mastitis is an inflammation of the mammary gland characterized by pathological changes in the udder and abnormal compositional or visible changes in the milk of dairy animals. The pooled prevalence of subclinical mastitis and clinical mastitis in dairy animals is reported to be 42% and 15% worldwide, respectively, and 45% and 18% in India, respectively (Krishnamoorthy *et al*., 2021) [9]. The disease can occur in either clinical or subclinical form. Clinical mastitis presents with overt inflammatory signs such as heat, pain, redness, and swelling in the udder, along with visible abnormalities in the milk, while subclinical mastitis, the more common form, shows no visible signs but leads to reduced milk yield and alterations in milk composition.

The disease causes an estimated annual economic loss of 100 trillion US dollars worldwide (Lal *et al*., 2022) [11]. The average case of clinical mastitis results in a total loss of 435 US dollars (Rollin *et al*., 2015) [16]. In India, a study over a decade ago reported an annual economic loss of Rs 7165.5 crores due to mastitis (Bansal and Gupta, 2009) [3], which now likely reflects even greater economic damage. These losses are primarily due to decreased milk production, the need to discard contaminated milk, rising medication costs, increased management costs, and the premature culling and replacement of affected animals.

Mastitis primarily results from intramammary infections, mainly caused by bacteria but occasionally by viruses, fungi, and algae (Krishnamoorthy *et al*., 2013, Cobirka *et al*., 2020; Ksouri *et al*., 2015,) [9, 5, 10]. It can also be induced by various factors, including injuries (Physical and chemical), toxic substances (e.g., Dicomural toxicity), allergies, and neoplasms (Knife Kibebew, 2017; Constable *et al*; 2017; Peek and Divers., 2018) [8, 6, 12].

Although various mastitis vaccines are commercially available, none of them provide sufficient protection and are not cost-effective at the same time (Sharun *et al*., 2021; Rainard *et al*., 2022, Tomanic *et al*., 2023) [17, 15, 20, 15, 20]. The short comings of vaccines have been attributed to low intensity of antigenic stimulus in subclinical cases, virulence and immune evasion tactics of pathogens, Dilution and quenching of defence factors and oxygen radicals by milk respectively, diversity of pathogenic isolates etc. (Reinard *et al*., 2022) [15]. Antibiotic therapy is the only solution for the disease but current Antibiotic based therapeutic interventions seem costly, risky and at times ineffective due to widespread resistance. The newer antibacterial drugs available against mastitis belong to third generation cephalosporins group considered highest priority critically important antimicrobials (HPCIA) for humans and discouraged for use in Farm animals (WHO 6th revision, 2018) [23].

Hence, early diagnosis of mastitis cases is of paramount importance owing to the current shortcomings of mastitis vaccines and therapeutics globally and the problem of antibiotic resistance. This study provides a comprehensive assessment of mastitis prevalence in dairy cattle in Kashmir, India, and highlights the importance of using multiple diagnostic methods to achieve accurate detection. The findings emphasize the need for regular screening and early intervention strategies to improve udder health and ensure the sustainability of the region's dairy sector

Materials and Methods

Ethical approval

Thermal Imaging and milk sampling were performed as per the guidelines of the National Dairy Research Institute (NDRI) Animal Ethical Committee for care and use of experimental animals.

Study Design and Sample Selection: This cross-sectional study was conducted in several districts of Kashmir, India, to investigate the prevalence of clinical and subclinical bovine mastitis. A total of 394 dairy cattle from both organised and unorganised farms across six districts of Kashmir were selected for examination. The sample size was calculated using prevalence rates from existing literature, with a 95% confidence level, using Fischer formula to ensure statistical significance, Random sampling and representativeness of the dairy cattle population in the region.

Sample Size Formula

The formula for calculating the sample size for estimating a population proportion in a large population is:

$$n=Z^2\times P\times(1-P)/E^2$$

Where n is the sample size, Z is the Z-value for the desired confidence level (1.96 for 95% confidence), P is the expected prevalence and E is the margin of error (5%).

Calculated Sample size for the study on the prevalence of mastitis across the various districts and blocks/tehsils in Kashmir were subjected to proportional allocation. Samples are distributed proportionally, based on the cattle population in each block or district, to ensure representation reflective of the actual population.

Inclusion and Exclusion Criteria

The study's inclusion criteria consisted of cross bred cows aged 2 years and above, of various dairy breeds such as Holstein and Jersey, and those in different stages of lactation. Conversely, the exclusion criteria excluded cows with concurrent diseases other than mastitis, as well as those currently undergoing treatment for mastitis.

Selection of Dairy Farms

Dairy units were randomly selected from each district and block to ensure a representative sample (Figure1).

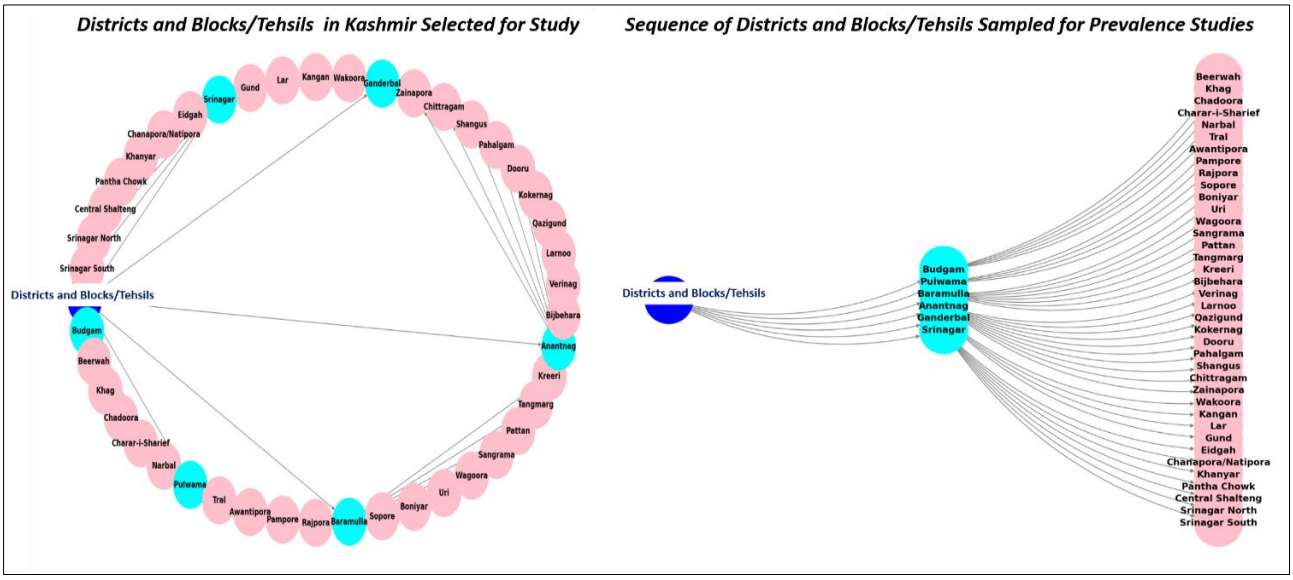

Fig 1: The image visualizes the geographic sampling strategy for the prevalence study, showcasing various districts of Kashmir and their respective blocks or tehsils where milk samples were collected

Thermal Imaging

Thermographic images were captured using a Teledyne FLIR E8XT WiFi infrared camera (Wilsonville, Oregon, USA). This camera features an auto-focus lens and can measure temperatures ranging from -20 °C to +550 °C. It boasts a 320 x 240 pixel resolution for both the detector and display, with a temperature accuracy of ±2% or ±2 °C, and a three-inch display screen. Images were taken at the udder

level, maintaining a minimum distance of 0.5 meters from each udder side to ensure a clear view for analysis. All thermographic images were collected right before milking commenced.

Milk Sampling and Analysis

Following the thermal imaging, milk samples were collected from cows in the specified districts and blocks/tehsils of Kashmir, JK (Figure 1) involving the following steps:

1. **Sample Collection:** Milk samples were collected aseptically from all four quarters of the udder of each cow. The udder was cleaned and disinfected before sampling to avoid contamination.
2. **Sample Handling:** Sterile containers were used for milk collection, and each sample was labelled with the farm, district, and block details.
3. **Transport and Storage:** The samples were transported to the laboratory on ice to prevent bacterial growth and preserve sample integrity.

Diagnostic tests

Approximately 10 mL of collected composite milk was used for diagnostic tests. These samples were immediately tested for the California Mastitis Test (CMT) score and somatic cell count using a De Laval automatic SCC Counter (Tumba, Botkyrka, Sweden. Composite milk samples with an SCC range from 200,000 to 1,200,000 cells per mililiter in crossbred cows were identified as positive for subclinical mastitis. Composite milk with a CMT score of 2 or higher and an SCC exceeding 1,200,000 cells per milliliter were classified as clinical cases (Lal *et al*., 2022) [11]. The samples were also subjected to Bacterial culture using standard laboratory techniques and selective media (Quinn *et al.*, 2002) [14]. Aseptically collected milk samples were cultured on selective media for mastitis-causing pathogens (Figure 3). Samples were incubated for 24-48 hours, and bacterial growth was identified and quantified according to standard microbiological procedures.

Prevalence was calculated as the number of positive cases divided by the total number of animals examined, expressed as a percentage. The efficacy of each diagnostic method was evaluated by comparing the number of cases detected to the total number of confirmed cases (based on SCC and clinical examination).

Results

Analysis of Mastitis Detection Methods in Kashmir, India

This prevalence study compared the performance of four diagnostic methods for detecting mastitis in 394 dairy cattle in Kashmir, India. The results revealed that each method has its strengths and weaknesses, highlighting the importance of a multi-faceted approach to mastitis diagnosis. The study employed four diagnostic methods to detect mastitis in dairy cows, each with its advantages and limitations. Somatic Cell Count (SCC) method used as standard test detected 141 cases, including 37 clinical and 104 subclinical (Figure 1), and is the most effective method for detecting subclinical mastitis, although it also requires specialized equipment and expertise. The California Mastitis Test (CMT) detected 85 cases, including 33 clinical and 52 subclinical, and is relatively simple and cost-effective, although its accuracy may be compromised for subclinical mastitis (Table 1). The prevalence of mastitis was found to be 35.80 percent in Kashmir (26.4% subclinical mastitis and 9.40% clinical mastitis) based on SCC (Figure 2).

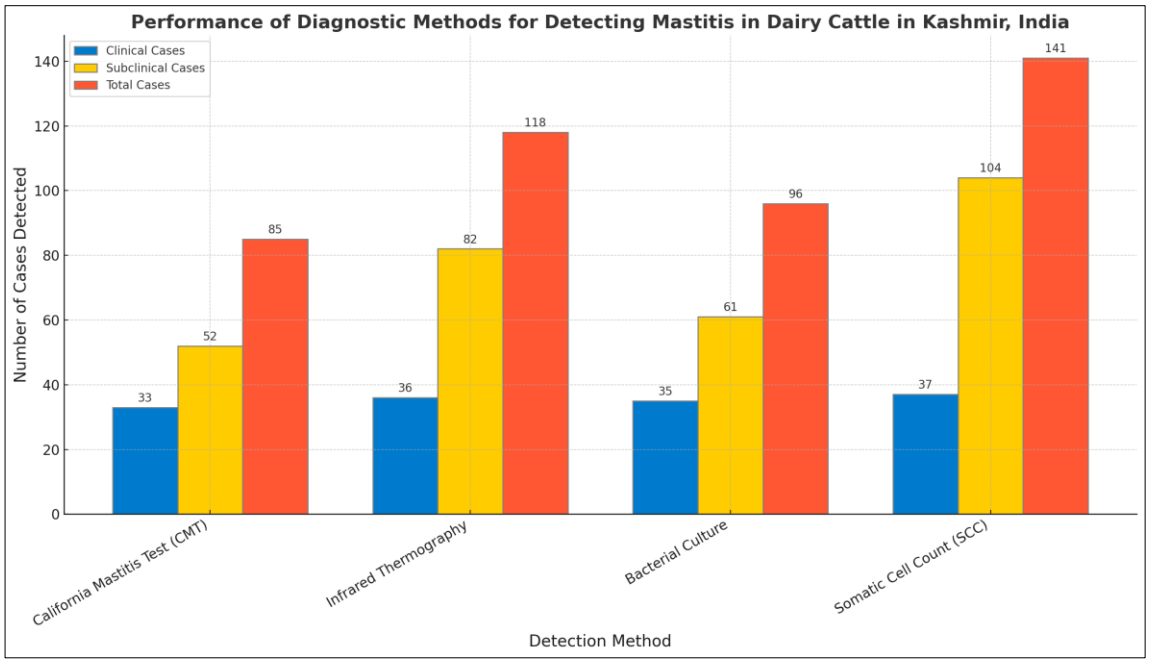

Fig 2: Bar graph showing total cases, subclinical cases and clinical cases detected in dairy cattle using different detection methods

Table 1: Performance of four diagnostic methods for detecting mastitis in 394 dairy cattle in Kashmir, India

Detection Method	Clinical Cases Detected	Subclinical Cases Detected	Total Cases Detected
California Mastitis Test (CMT)	33	52	85
Infrared Thermography	36	82	118
Bacterial Culture	35	61	96
Somatic cell count (SCC)	37	104	141

Bacterial culture and Pathogen Identification

Out of 141 samples detected positive by SCC method, Bacterial pathogens were isolated successfully from 98 (68%) samples on selective media (Figure 3). The most common pathogens isolated from mastitis cases were mainly Staphylococcus aureus (73%) and Escherichia coli (24%). Other pathogens detected included Klebsiella species (3%) (Figure 4).

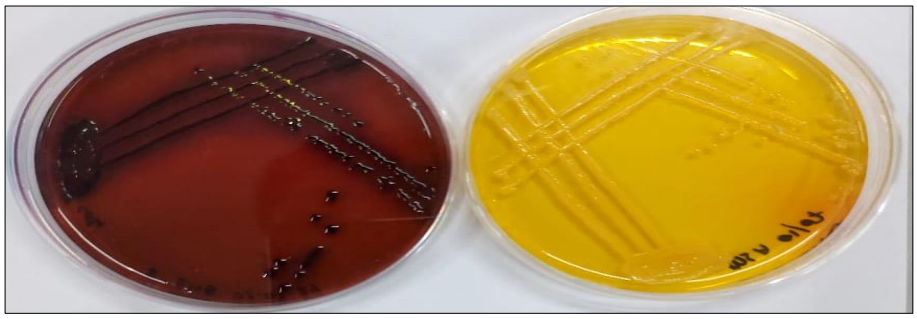

Fig 3: The image displays two agar plates: an Eosin Methylene Blue (EMB) plate (Left) showing metallic sheen with dark colonies indicative of *E. coli*, and a Mannitol Salt Agar (MSA) plate(Right) where yellow colonies suggest mannitol fermentation by *Staphylococcus aureus*

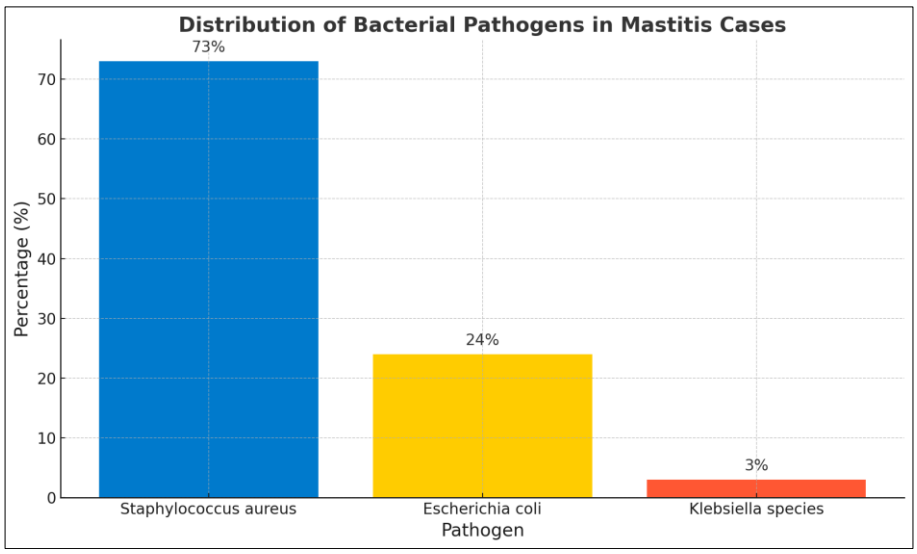

Fig 4: Bar graph representing the distribution of bacterial pathogens isolated from mastitis cases. The graph shows the percentages of *Staphylococcus aureus, Escherichia coli, and Klebsiella* species among the isolated pathogens

Infrared thermography

It examines the udder skin surface temperature (USST) in crossbred cows, focusing on the differences between healthy and infected quarters under varying ambient temperatures while maintaining a constant core body temperature. The study findings reveal distinct temperature patterns in udder quarters based on health status. For healthy udder quarters, the average Udder Surface Temperature (USST) at an ambient temperature of 30 °C was 37.10 °C, with a standard deviation of ±0.2 °C, indicating a slight increase that correlates with environmental conditions and reflects the body's thermoregulatory response. In contrast, infected udder quarters exhibited higher temperatures: subclinical cases had an average USST of 37.90 °C (±0.4 °C) (Figure 5), significantly elevated due to the inflammatory response, while clinical cases showed an even greater average USST of 38.20 °C (±0.2 °C), suggesting a more severe inflammatory reaction associated with the progression of the infection. Infrared Thermography detected 118 cases, including 36 clinical and 82 subclinical, and is effective at detecting subclinical mastitis, but requires specialized equipment and expertise.

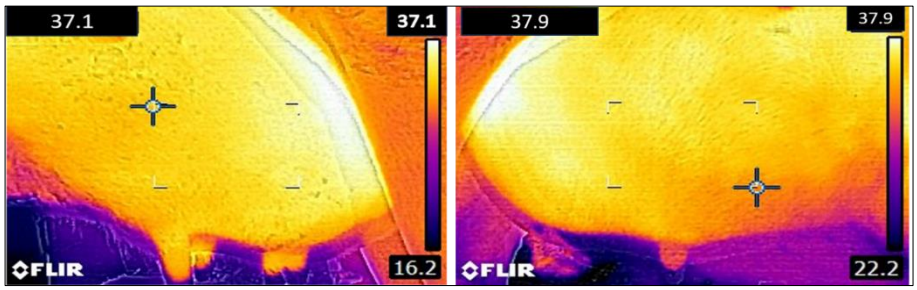

Fig 5: The image illustrates the use of infrared thermography to detect temperature variations in the udder of a cow, comparing a normal udder (left: Temperature 37.1 °C) against one affected by mastitis (Right- Temperature 37.9 °C)

The sensitivity, specificity, true positives (TP), false negatives (FN), true negatives (TN), and false positives (FP) for each test was calculated as shown in table 2.

Accuracy: With the total actual Mastitis cases being 141 as detected by somatic cell count, the accuracies for other detection method are as follows: The California Mastitis Test (CMT) has an accuracy of 60.28%, Infrared Thermography reaches 83.69%, and Bacterial Culture shows an accuracy of 68.09%. These percentages indicate the effectiveness of each method in detecting cases of mastitis relative to the total known cases.

Table 2: Shows the True positives (TP), False negatives (FN), True negatives (TN), Sensitivity and Specificity of each Test

Detection Method	True Positives (TP)	False Negatives (FN)	True Negatives (TN)	Sensitivity (%)	Specificity (%)
California Mastitis Test (CMT)	85	56	253	60.28	76.0
Infrared Thermography	118	23	253	83.69	78.0
Bacterial Culture	96	45	253	68.09	89.0
Somatic Cell Count (SCC)	141	0	253	100.0	100.0

This table provides a clear comparison of the effectiveness of each method in detecting mastitis, showcasing both the sensitivity and specificity of each testing approach (Figure 6 &7)

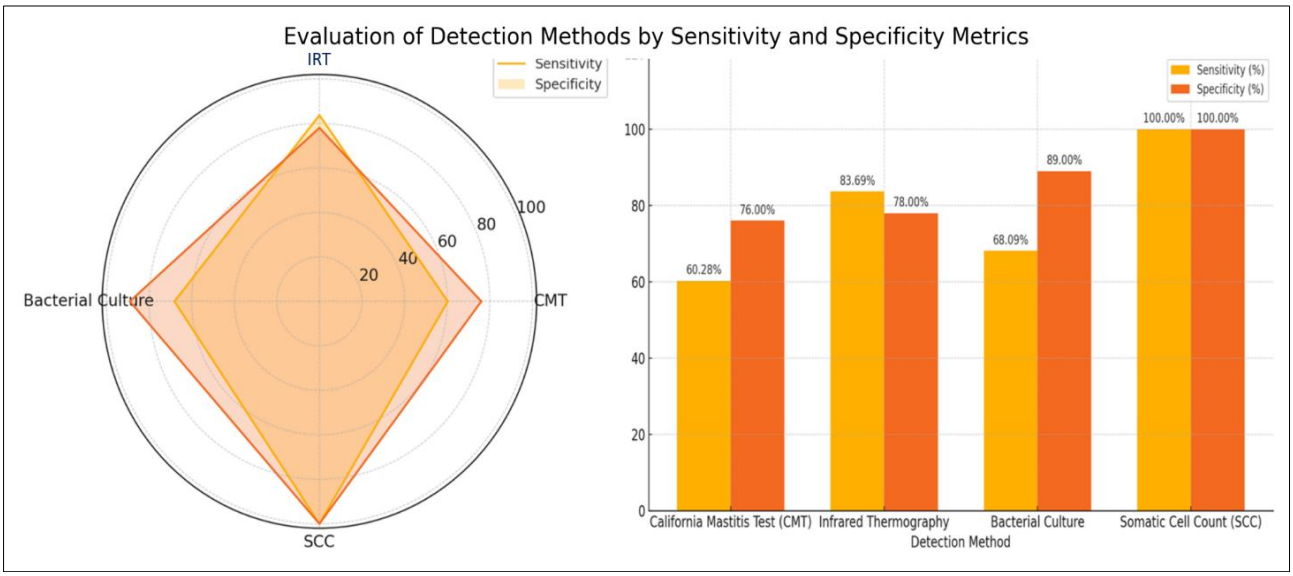

Fig 6: The image displays the sensitivity and specificity of various detection methods for dairy cattle udder health conditions, presented through both a radar chart and a bar chart. The radar chart provides a visual comparison, while the bar chart quantifies the metrics for California Mastitis Test, Infrared Thermography, Bacterial Culture, and Somatic Cell Count

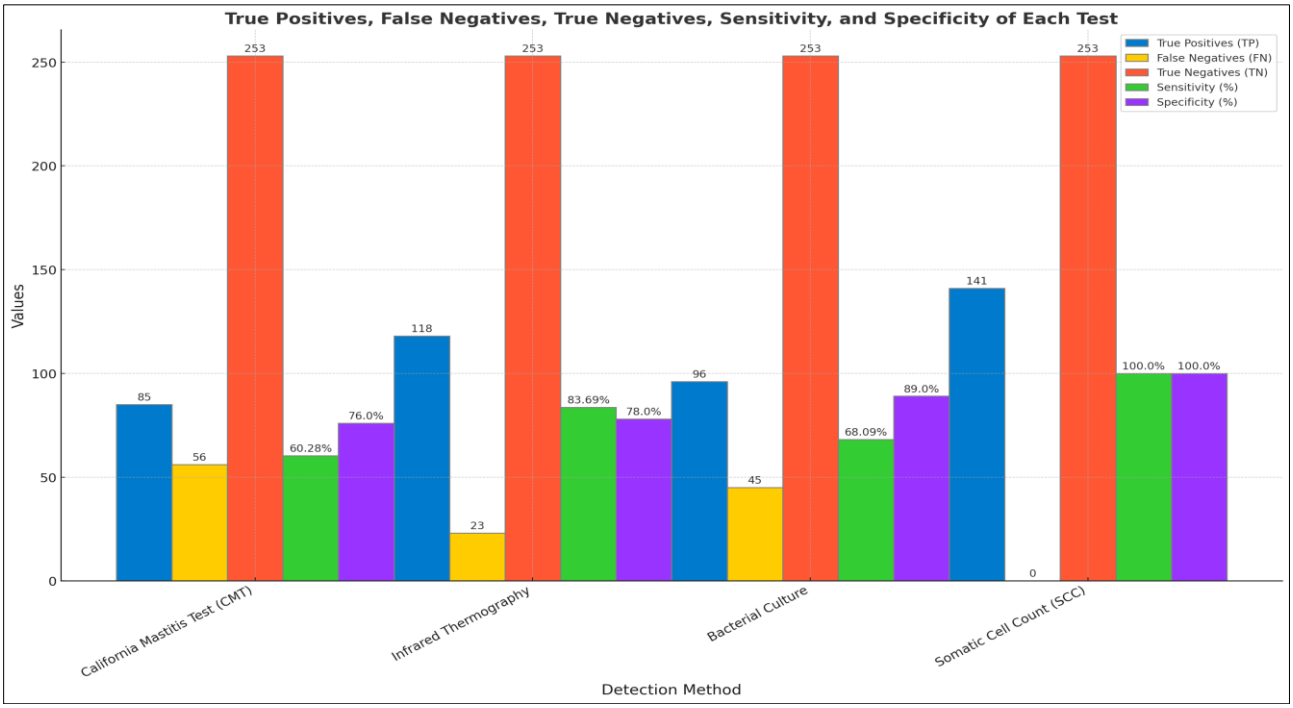

Fig 6: Graphical representation of The sensitivity, specificity, true positives (TP), false negatives (FN), and true negatives (TN) for each test

Risk Factors

Analysis of risk factors revealed that poor udder hygiene, inadequate milking practices, and lack of proper nutrition were significantly associated with higher incidence and prevalence of mastitis. Farms with better management practices showed lower rates of infection**.**

Statistical Analysis

The SCC (Somatic cell count method) is considered the gold standard for detection, with an accuracy of 100%. Infrared Thermography emerges as the most accurate among the other methods, with an accuracy of 83.69%, Bacterial culture method has 68% sensitivity while CMT test has the lowest accuracy at 60.28%, indicating it is the least reliable method overall. The Chi-square test for independence reveals a significant difference in the detection rates among the four methods. The null hypothesis, which states that there is no significant difference in the detection rates, is rejected, as the Chi-square statistic of 23.76 and the p-value of less than 0.0001 suggest a statistically significant difference in the detection rates among the four methods. Additionally, among 96 bacterial isolates, the pathogen distribution analysis shows a predominance of Staphylococcus aureus (70 isolates), followed by Escherichia coli (23 isolates) and Klebsiella species (3 isolates). The chi-square goodness-of-fit test (χ^2 = 91.94, Degree of freedom:2, $p< 0.0001$) indicates this distribution is significantly different from what would be expected by chance, highlighting the importance of targeting these specific pathogens in mastitis prevention and treatment strategies.

Discussion

Mastitis remains a significant challenge in dairy farming globally, impacting both the health of dairy cows and the profitability of dairy operations. Early diagnosis is important to curtail the infection. For the effectiveness of different mastitis detection methods, it's crucial to consider the sensitivity and specificity of each technique, as revealed by the data collected from testing 394 milk samples.

The Somatic Cell Count (SCC) method demonstrated the highest effectiveness, achieving 100% in both sensitivity and specificity. This aligns with research that highlights SCC as a highly reliable indicator of mastitis, particularly in identifying subclinical infections that might not be detectable through physical symptoms alone (Smith *et al*., 2018) [19]. The complete absence of false negatives and false positives in this method underscores its precision and reliability in field conditions.

Infrared Thermography also showed high sensitivity at 83.69%, indicating its strong capability in identifying mastitis cases, particularly in the early stages when physical symptoms are not yet prominent. This method's high sensitivity makes it an excellent tool for early detection, which is crucial for effective management and treatment, reducing potential losses in dairy production.

These findings underscore the utility of measuring USST as a diagnostic tool for detecting mastitis in dairy cows. The elevated temperatures in infected quarters, regardless of the stage of infection, reflect the physiological changes associated with inflammation. This method provides a non-invasive way to screen for udder health, enabling early detection and management of mastitis, which is crucial for maintaining animal welfare and productivity in dairy operations.

Statistical analysis further supports these findings. The chi-square test for independence (χ^2 = 23.76, $p< 0.0001$) strongly indicates significant differences in detection rates among the four methods. Additionally, the pathogen distribution analysis shows a predominance of Staphylococcus aureus (73%) among the isolates, with the chi-square goodness-of-fit test (χ^2 = 91.94, $p< 0.0001$) confirming a highly significant non-uniform distribution of pathogens.

The comparison of different mastitis detection methods reveals significant variations in their performance. Infrared Thermography shows promise with high sensitivity (83.69%) but has the lowest specificity (78%), suggesting it may produce more false positives. The California Mastitis Test (CMT) and Bacterial Culture showed lower sensitivity at 60.28% and 68.09%, respectively California Mastitis Test (CMT) performs the poorest overall, with the lowest sensitivity (60.28%) and accuracy (60.28%), indicating it misses a substantial number of positive cases and is the least reliable method. Overall, the methods indicate high prevalence of mastitis in Kashmir.

Previous studies have also highlighted a high prevalence of mastitis in certain regions and farms in Kashmir. Tufani *et al*. (2012) [21] reported an overall prevalence of clinical mastitis of 8.08% in Kashmir. Dar *et al*. (2014) [7] found the prevalence of subclinical mastitis in cows to be 51.61% on a quarter basis in J&K. Sharma *et al*. (2012) reported the prevalence of subclinical mastitis as 42.18% on an animal basis and 19.14% on a quarter basis in the Jammu region. Bhat *et al*. (2017) [4] observed the incidence of clinical mastitis in bovines of the Jammu region to be 11.5% on an animal basis and 5.76% on a quarter basis. Qadri *et al*. (2017) [13] reported the prevalence of subclinical mastitis as 45% in crossbred Jersey cows at the Mountain Livestock Research Institute (MLRI) in Mansbal. Ashraf *et al*. (2018) [2] found the prevalence of subclinical mastitis in cattle from the Kashmir Valley during the winter season to be 47.58% on an animal basis and 26.67% on a quarter basis. Ali *et al*. (2021) [1] reported an overall prevalence of subclinical mastitis in crossbred Holstein-Friesian cows to be 81.48% in the Ganderbal district.

The high prevalence of bovine mastitis in Kashmir necessitates improved management strategies. With over one-third of dairy cattle affected, targeted antibiotic therapy for Staphylococcus aureus and E. coli, along with enhanced hygiene practices, are crucial. Infrared Thermography is the most effective method for detecting subclinical mastitis, and a multi-method approach is recommended for accurate diagnosis. Regular screening and early intervention are vital to improve udder health and reduce economic impact. Comprehensive control programs, including vaccination and farmer education, are essential for sustainable dairy production.

Further research is needed to investigate the cost-effectiveness of different diagnostic methods, evaluate the impact of mastitis control programs, and explore novel diagnostic tools and technologies for mastitis detection. This study provides valuable insights into the prevalence and diagnosis of mastitis in Kashmir and underscores the need for tailored control strategies to ensure sustainable dairy production.

Conclusion

This study of mastitis prevalence in Kashmir, India, reveals significant variations in the effectiveness of different diagnostic techniques. The Somatic Cell Count (SCC) method proved most accurate with perfect sensitivity and specificity, particularly for subclinical cases. Infrared Thermography showed high sensitivity, while Bacterial Culture was valuable for pathogen identification, highlighting the prevalence of Staphylococcus aureus. The California Mastitis Test, though simple, was the least reliable. These findings highlight the importance of a multi-faceted approach to mastitis detection, balancing accuracy with practical and economic considerations. This research provides a foundation for improving animal health, milk quality, and economic outcomes in the dairy industry, and points to future areas for investigation, including cost-benefit analyses and novel diagnostic technologies.

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Conflict of Interest

The authors declare that there are no conflicts of interest associated with this work.

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