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Original Research Article

Evaluation of the California mastitis test, pH and milk colour as indicators for subclinical mastitis detection in local dairy cows

Ramzi Amran*, Ahmad Alhimaidi, Aiman Ammari
Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

For correspondence:* ***Email:* *ramran@ksu.edu.sa*

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Abstract

Purpose: *To evaluate the utilization of diagnostic tools such as the California Mastitis Test (CMT) and milk pH and colour assessments for detecting subclinical mastitis (SCM).*
Method: *A total of 60 milk samples were collected from 60 apparently healthy local dairy cows in Dhamar City, Yemen, to investigate the incidence of SCM. The samples were subjected to California Mastitis Test (CMT) as well as tests for pH and colour.*
Results: *According to CMT results, 45 % of the cows had SCM. The rate of prevalence in (+), (++) and (+++) CMT results was 17 (28 %), 7 (12 %) and 3 (5 %), respectively. In terms of milk pH, 27 (45 %) of the samples had a pH between 6.6 and 6.8, indicating no signs of SCM. However, 20 (33 %) of the samples exhibited a pH < 6.6, reflecting increased acidity, while 13 (22 %) had a pH > 6.8, indicating alkaline milk, a marker of severe inflammation and tissue damage. The assessment of milk colour showed that 34 (57 %) of the samples had normal white milk, indicating no mastitis, while 17 (28 %) were yellow, suggesting mild to moderate inflammation or infection. Finally, 9 (15 %) of the samples were pink, indicating the presence of blood and severe inflammation with udder damage.*
Conclusion: *These findings underscore the reliability of CMT, pH and milk colour assessments as effective diagnostic tools for detecting SCM in dairy cows, thus providing valuable insights for early intervention and management strategies.*

Keywords: *Mastitis, Milk, California test, pH, Cows*

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INTRODUCTION

Mastitis is one of the most prevalent production diseases in cows globally and a leading cause of significant economic losses in dairy farms worldwide. It is recognized as the most frequent issue faced by dairy farms, impacting the profitability of the milk and dairy industries in both developed and developing countries [1]. However, developing countries are particularly susceptible to greater production challenges and financial losses due to the widespread occurrence of the disease [2]. Contributing factors may include inadequate nutrition, improper breeding practices, poor farm infrastructure and ineffective treatment strategies [3]. Early detection of mastitis is crucial for successful treatment and minimizing economic losses. These losses include the exclusion of milk from affected udders due to microbial contamination or antibiotic residues, increased treatment costs and premature culling or death of

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cattle due to the disease. Additionally, contamination of milk with pathogens can impact the health of suckling calves and may even facilitate transmission of pathogens to humans [4].

Mastitis in cows is classified into two types namely clinical and subclinical mastitis. Clinical mastitis is characterized by a sudden onset, leading to a decrease in milk production, along with physical, chemical and microbiological changes in the milk, as well as histopathological changes in the affected udder. These changes are evident as redness, heat, swelling and pain of the udder. In contrast, subclinical mastitis (SCM) lacks these visible symptoms. However, SCM is associated with cytochemical and bacteriological changes in the milk, which can be detected using field tests such as the California Mastitis Test (CMT), pH test and milk colour assessment [5].

The CMT is one of the most effective and widely used field tests for detecting SCM. It is affordable, simple to use, provides quick results and can be performed by the person handling the milking process without the need for specialized laboratories or equipment [6]. The normal pH of milk ranges from 6.6 to 6.8, but during mastitis, the pH rises as the milk becomes more alkaline. Inflammation of the mammary gland increases the permeability of blood capillaries, allowing alkaline blood components such as sodium and bicarbonate ions to enter the milk. Consequently, the pH may rise, reaching close to 7 in cases of severe clinical mastitis. This increase in milk pH is positively correlated with the severity of inflammation in the udder, resulting in the mixing of milk with blood and extracellular fluid components [7]. Milk colour is a valuable visual indicator for detecting mastitis in dairy animals, especially for farmers and veterinarians who monitor the physical characteristics of milk. Discoloration is often linked to infection or mastitis. During mastitis, milk may change from its usual white or creamy appearance to abnormal colours. Yellowish milk may result from the presence of pus or an increase in leukocytes (white blood cells) due to inflammation. Watery or bluish milk suggests a reduction in fat content, which can occur during mastitis, while pink or reddish milk indicates blood from udder tissue damage and ruptured blood vessels [8]. This study aimed to estimate the percentage of SCM in dairy cows in Dhamar City, Yemen, using a California test, a pH test and milk colour evaluation.

EXPERIMENTAL

Design

Milk samples were collected from 60 Zebu dairy cows aged between 4 and 7 years in Dhamar City, Yemen. Each sample underwent three different diagnostic tests to detect subclinical mastitis (SCM) namely; California Mastitis Test (CMT) [9] to assess the presence of somatic cells in milk; pH test conducted using a digital pH meter to identify any deviations from the normal pH range of milk and Milk Colour Evaluation, visual inspection to detect any discoloration, which may be indicative of infection or inflammation. These tests were applied to each sample and the results were analyzed to assess the reliability and efficacy of the methods in identifying subclinical mastitis in local dairy cows.

Milk sampling

The cow's breeder collected the milk samples to test for mastitis. The collection of milk samples from Zebu dairy cows involved first cleaning the udder with clean water and drying it to ensure sterility. The initial stream of milk was discarded and the subsequent milk was collected in 25 mL tubes. The samples were then stored in a cool box at 4 – 6 °C to prevent bacterial growth and preserve the milk until it reached the laboratory.

California Mastitis Test (CMT)

Milk samples were tested using the CMT reagent (CMT; Bovi-Vet, Kruuse, Germany) according to the manufacturer’s instruction by mixing 2 mL of milk with 2 mL of the CMT reagent. The mixture was shaken for 10 – 15 seconds. The reaction results were observed in a well-lit area and milk samples were checked for the presence or absence of clots or sediment. The results were based on the CMT score as follows; Negative (-) indicates no visible reaction, clots or sediment formation. This shows a low somatic cell count, meaning the milk is of very good quality and the cow is healthy; Weak positive (+) shows slight reaction, with minimal sediment or slight thickening of the mixture. This indicates a mildly elevated somatic cell count, suggesting a possible mild infection; Distinct positive (++) indicating moderate reaction, with visible sediment or thickening but no gel formation. This shows a moderately high somatic cell count, suggesting the presence of SCM; Strong positive (+++) indicates a strong reaction, with noticeable gel formation or clots. This infers a very high somatic cell count and a severe infection, meaning the milk is of poor quality and the cow has significant mastitis [9].

Milk pH

The pH of the milk was determined using a digital pH meter (pH 211, Hanna Instruments, Portugal). Fresh milk samples were stirred and a clean electrode was inserted to determine the pH at room temperature (20 – 25 °C). Each sample was tested three times and the average pH value was recorded. Dairy cows with milk pH > 6.8 were classified as having subclinical mastitis, while those with a pH < 6.6 were considered healthy [10].

Milk colour

Milk colour changes were assessed using a visual sensory test to detect SCM. Fresh milk samples were stirred to ensure uniformity and the evaluation was conducted at room temperature (20 – 25 °C) in a well-lit area [11].

Statistical analysis

Descriptive analysis of CMT, pH and milk colour was performed using GraphPad Prism 8.

RESULTS

Findings from California Mastitis Test (CMT)

The CMT results of dairy cows showed that 33 (55 %) of the samples were negative (-), indicating no mastitis, 17 (28 %) had weak positive results (+), suggesting early or mild inflammation while 7 (12 %) samples showed distinct positive (++), indicating a moderate infection that likely requires attention. Lastly, 3 (5 %) had strong positive results (+++), pointing to severe SCM requiring immediate intervention (Figure 1).

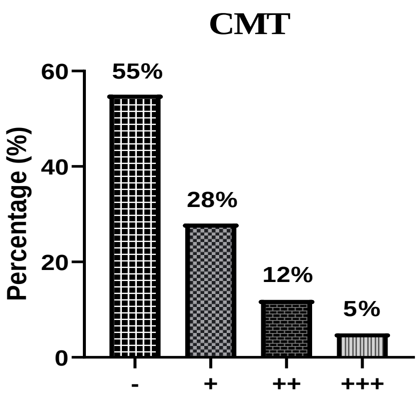

Figure 1: CMT results as indicators of SCM severity in dairy cows. (-): Negative; (+): Weak positive; (++): Distinct positive; (+++): Strong positive

Milk pH

Results show that 27 (45 %) of the milk samples had a pH between 6.6 and 6.8, indicating no signs of SCM, as the milk pH remained within the normal range. Meanwhile, 20 (33 %) of the samples showed a pH < 6.6, reflecting increased acidity, which suggests a progression of infection or significant udder damage, likely due to bacterial activity. Lastly, 13 (22 %) of the samples had a pH > 6.8, indicating alkaline milk, a sign of severe inflammation and tissue damage in the udder (Figure 2).

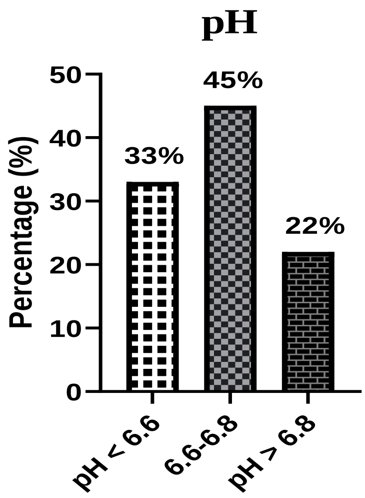

Figure 2: Milk pH variability as an indicator of SCM in dairy cows

Milk colour

The milk's colour results as presented in Figure 3 shows that 34 (57 %) of the samples had normal white milk, indicating no mastitis. Samples with yellow colour were 17 (28 %), suggesting mild to moderate inflammation or infection, with signs of an immune response in the udder. Furthermore, 9 (15 %) of the samples were pink, indicating the presence of blood and severe udder damage.

DISCUSSION

Mastitis is a disease primarily impacting dairy cows, resulting from a bacterial infection in the udder glands. Subclinical mastitis (SCM) can only be detected through a laboratory somatic cell count test, as there are no visible changes in the udder [1,4]. Contributing factors to mastitis include poor barn sanitation, inadequate hygiene and improper milking management. The condition leads to reduced milk production and quality, ultimately resulting in financial losses for farmers [2].

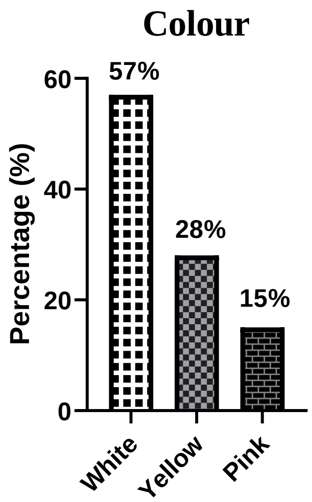

Figure 3: Milk colour variability as an indicator of SCM in dairy Cows

The California mastitis test (CMT) is an effective early detection method for identifying SCM that can be conducted directly in the field. This test utilizes a paddle and specific reagents to assess the severity of the condition. Higher results from the CMT test indicate an increased number of somatic cells in the milk [12].

The results of the CMT revealed that out of 60 milk samples, the number of samples that showed Negative (-), Weak positive (+), Distinct positive (++), and Strong positive (+++) were 33 (55 %), 17 (28 %), 7 (12 %), and 3 (5 %) respectively. Another study noted almost identical outcomes of 43.1 and 46 % [13]. In contrast, Rochmah *et al,* [14] reported lower findings of 25 and 35.5 %, respectively, while Meskini *et al,* reported higher findings of 59.68, 50.5, 62.8 and 81.48 %, respectively [15]. The differences in prevalence amongst studies might be due to differences in milking practice, environmental conditions, factors relating to animals, causative organisms and animals' immune status [16].

An elevated pH in milk from cows with SCM has also been documented by other studies [17]. This increased alkalinity/pH in SCM cases is thought to result from greater permeability of blood capillaries due to mammary gland inflammation, allowing alkaline blood components such as sodium (Na^+) and bicarbonate ions to enter the milk [7]. This, combined with reduced lactose production by the gland, resulted in milk pH rising above 7.0 [18]. In this study, the average pH values for healthy and SCM milk were 6.30 ± 0.06 and 6.90 ± 0.05, respectively. These findings align with a previous report [19] that also observed elevated pH levels in mastitic milk. The increase in pH during SCM may be attributed to greater membrane permeability, leading to the leakage of salts and various ions into the milk [20]. Additionally, the rise in milk leukocyte concentration could contribute to the higher pH values [21].

Furthermore, early detection and management of SCM are crucial for maintaining milk quality and herd productivity. Cows with SCM often show no visible signs of illness, making it imperative for dairy farmers to adopt regular monitoring practices, including visual assessments of milk, to identify potential problems before they escalate. The economic impact of mastitis on dairy production is significant, as it can lead to reduced milk yield, increased veterinary costs, and compromised milk quality, ultimately affecting profitability [22].

CONCLUSION

The California mastitis test identifies subclinical mastitis in 45 % of the cows. Milk pH analysis reveals abnormal pH levels in 33 % of samples. Additionally, visual inspection for milk colour changes is effective in 43 % of cases. These findings highlight the effectiveness of the CMT, pH and milk colour assessments as reliable indicators for detecting SCM in dairy cows, thereby offering crucial insights for early intervention and management strategies.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Conceptualization; Ramzi Amran, Aiman Ammari, Ahmad Alhimaidi; methodology, Ramzi Amran, Aiman Ammari; software, Ramzi Amran; validation, Ramzi Amran; formal analysis, Ramzi Amran; investigation, Ahmad Alhimaidi; resources, Ramzi Amran; data curation Ramzi Amran, Aiman Ammari; writing—original draft preparation, Ramzi Amran, Aiman Ammari; writing—review and editing, Ramzi Amran, Aiman Ammari and Ahmad Alhimaidi; visualization, Ahmad Alhimaidi; supervision, Ahmad Alhimaidi; funding acquisition, Ahmad Alhimaidi. All authors have read and agreed to the published version of the manuscript.

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