

# Comparative prevalence assessment of subclinical mastitis in two crossbred dairy cow herds using the California mastitis test

## Abstract

The study was conducted on two herds of Jersey crossbred dairy cows. Samples were taken from two dairy farms to conduct the California Mastitis Test (CMT) during milking. A total of 115 animals were sampled, with 65 animals from Farm 1 and 60 animals from Farm 2. Milk samples were collected from all four quarters of each cow. The study analyzed a total of 460 quarters from the collected milk samples. Among these quarters, 132 quarters (28.69%) were found to be infected. The prevalence of mastitis was 45% in Herd 1 and 25% in Herd 2. The study found that only one quarter of the udder was more affected in both herds. The CMT score (likely a measure of mastitis severity) increased during late lactation. Infected cows had an average milk yield/day of 3.6 liters. Inflammatory (within or under the mammary gland) infections were more common in the first and second parity cows (cows that had given birth to one or two calves, respectively). This could be ascribed to the fact that dairy cows in their starting lactations are more vulnerable to the environmental pathogens because they might not have built up their immunity yet. Overall, this study provides insights into the prevalence, severity, and patterns of mastitis in Jersey crossbred dairy cows located at two different farms. The study highlights factors such as the stage of lactation, parity, and herd differences that can influence the occurrence and severity of mastitis in these animals.

**Keywords:** cattle, dairy farm, subclinical mastitis, prevalence and California mastitis test

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## Introduction

Mastitis is a costly and widespread disease with various implications on milk production, food safety, veterinary expenses, and overall animal health. Disease that affects dairy cattle globally. Costs associated with mastitis include reduced milk production, milk condemnation due to antibiotic residues, veterinary expenses, culling of chronically infected cows, and occasional deaths. Moreover, there is a potential zoonotic risk associated with mastitis, as bacteria and toxins can be shed into milk, posing a threat to human health.

Mastitis is caused by a range of pathogens and can be classified into contagious and environmental forms. Contagious mastitis is spread from cow to cow during milking and often leads to chronic sub-clinical infections with occasional flare-ups of clinical symptoms. Mastitis can be categorized as clinical (easily detectable with visible signs) or sub-clinical (no visible signs but reduced milk production and increased somatic cell count). The incidence of mastitis varies across parities, stages of lactation, and species of animals. A significant proportion of affected animals experience mastitis within a specific period of lactation (30-90 days). Crossbred cows are observed to have a higher incidence of mastitis, indicating their susceptibility to the disease. The research introduces the objective of a specific study, which is to determine the prevalence of subclinical mastitis in an organized dairy farm.<sup>1</sup> Overall, the research emphasizes the widespread impact of mastitis on dairy cattle, its economic and health-related consequences, and the need for research and management strategies to address this significant issue. The study's objective of assessing the prevalence of subclinical mastitis in a specific dairy farm underscores the importance of understanding and managing this disease within localized contexts.

## Materials and methods

This study focused on Jersey crossbred dairy cows and aimed to investigate mastitis prevalence. The study was conducted on two herds of crossbred Jersey dairy cows. Data and samples were collected from 65 animals from Farm 1 and 60 animals from Farm 2. The California mastitis test (CMT) was performed on the milk samples during morning and evening milking. Milk samples were collected from all four quarters of each cow. The data collection period extended from January 2019 to May 2019 (the average lactation days in both herds were around 300). The study was conducted at two specific locations in Palampur Himachal Pradesh, India. The prevalence studies likely involved calculating the proportion of cows with mastitis in each herd and determining the overall prevalence based on the collected samples. All statistical analyses were carried out using Microsoft Excel (2010). The data collected, including prevalence calculations and Chi-square tests, were managed, and analysed using Excel spreadsheets. The study followed animal ethics guidelines outlined by the institute to ensure the ethical treatment of animals involved in the research. The data collection, analysis, and ethical considerations were all outlined in the provided description (Table 1).

## Results and discussion

### Prevalence of sub-clinical mastitis (SCM)

The total number of dairy cattle that were included in the screening process, which is 125 animals in this case (Table 2). The number of dairy cattle that were found to be affected (presumably with mastitis or a similar condition) were 44 cows representing 35.2% of the whole sample. Early intervention at the subclinical

stage can prevent the development of full-blown clinical mastitis, leading to reduced economic losses. There are various factors influencing mastitis prevalence. Differences in mastitis prevalence among breeds could be due to behavioural habits and variations in udder and teat development among cows.<sup>2</sup> Variation in prevalence can also be attributed to different regions, breeds, therapeutic practices, management conditions (especially milking management), hygiene practices, care of teat injuries, presence of microorganisms in the environment, and the effectiveness of mastitis control programs. The study's results are supported and found a similar prevalence of subclinical mastitis (42.2 percent) in Uruguay.<sup>1</sup> The current study findings differ from those.<sup>3,1</sup> These later studies reported higher prevalence rates of subclinical mastitis ranging from 75.9 to 89.54 % in Tanzania and Ethiopia, respectively.<sup>4</sup> Overall, the prevalence of sub-clinical mastitis is important of detecting and managing subclinical mastitis to prevent the progression to clinical mastitis. The prevalence of SCM was highest in lactating cows having third lactation and high yielding (cows produced >10-liter milk per day) both in local breed and crossbred cows.<sup>5</sup> The prevalence of SCM can vary widely depending on the population studied, diagnostic methods employed, and geographic location. Sub-clinical mastitis (SCM) is a prevalent and economically significant condition in dairy cattle worldwide. Despite its subtle clinical presentation, SCM can have a profound impact on milk quality and production, leading to substantial economic losses for dairy farmers. This research paper aims to discuss the prevalence of SCM, its risk factors, detection methods, and potential management strategies.

**Table 1** Interpretation of California mastitis test

SL No	CMT Score	Description	Interpretation
1	0	No slime or gel formation	Healthy Quarter
2	+	Mixture appearance or gel-like	SCM (Sub clinical mastitis)
3	++	Mixture distinctly forming gel	SCM (Sub clinical mastitis)
4	+++	Mixture thickened immediately and tends to form jelly	Serious mastitis

**Table 2** The prevalence of sub-clinical mastitis in dairy cattle

Animals	No. of animals screened	No. of animals affected	Percentage
Dairy Cattle	125	44	35.20%

Bovine mastitis, driven by microbial infection and genetic factors, poses a significant health threat to cows and dairy product safety, with recent research highlighting the emerging role of long non-coding RNAs (lncRNAs) in inflammation and offering potential avenues for molecular breeding and therapeutic strategies.<sup>6</sup>

The prevalence of SCM in dairy cows was 37.7% during the chosen periods, demonstrating a slight rise in the disease's occurrence when compared to the years 2012 to 2016 and 2017 to 2021.<sup>7</sup> In the seven regions, the estimated prevalence of SCM ranged from 36.4% to 50.2%, with no statistically significant variation. The Inner Mongolia Autonomous Region had the highest prevalence of SCM at 72%, while Hubei Province had the lowest prevalence at 19%.<sup>8</sup> In Finland, the prevalence of subclinical mastitis has declined in recent decades from 22.3% in 1991 and 20.1% in 2001 to 19.0% in 2010.<sup>5</sup> Based on the somatic cell count threshold of  $\geq 200,000$  cells/ml, the estimated SCM prevalence in 2012 in Sweden was 25.7% 21.0% in Norway,<sup>9</sup>

and 26.0% in Denmark.<sup>10</sup> Using a similar, it was reported that the prevalence of mastitis in huge Estonian herds was as high as 52.7%.<sup>11</sup>

Overall, the prevalence of sub-clinical mastitis is important of detecting and managing subclinical mastitis to prevent the progression to clinical mastitis.

### Quarter-wise prevalence of subclinical mastitis

Table 3 provides detailed information about the screening and prevalence of SCM based on quarters (mammary glands) among dairy cattle. The specific quarter in mammary gland being referred to; as LF (Left Front), LH (Left Hind), RF (Right Front), and RH (Right Hind). Number of quarters screened; this indicates the total number of quarters from dairy cattle that were included in the screening process. Number of quarters functioned; this specifies the number of quarters that were functioning normally. Absence of SCM (%); this shows the percentage of quarters that were deemed to be free from subclinical mastitis (SCM). Sore of CMT (%); this provides the breakdown of the California Mastitis Test (CMT) scores for each quarter. The CMT scores are categorized as follows: 0: Sore of CMT (%) - No reaction to the CMT test. +: Sore of CMT (%) - Slight reaction (low positive) to the CMT test. ++: Sore of CMT (%) - Moderate reaction to the CMT test. +++: Sore of CMT (%) - Strong reaction (high positive) to the CMT test. Total positive (%); this represents the total percentage of quarters that showed positive reactions to the CMT test, indicating the presence of subclinical mastitis. Blind Quarter (%): This shows the percentage of quarters that were considered "blind" (not functional) due to the presence of severe mastitis. The pattern of clinical mastitis occurrence was reported to be 26.94%. Among the quarters (mammary glands), the highest involvement was observed in the right front (RF) quarters, with a prevalence of 36.88%. This was followed by the right hind (RH), left hind (LH), and left front (LF) quarters. Crossbred cows exhibited a higher incidence of clinical mastitis in forequarters compared to buffaloes. Among the forequarters, the right forequarters were found to be more susceptible, which is consistent with the findings of.<sup>12</sup> The prevalence of clinical mastitis found in this study (26.94%) was lower than that reported by<sup>13</sup>(46.6%) and <sup>10</sup> 55.5%). The incidence of mastitis in dairy buffaloes was observed to be greater during the rainy season, aligning with the findings of.<sup>14</sup> Subclinical mastitis is a significant problem among the herds. Screening tests like the California mastitis test (CMT) along with other diagnostic methods can play a role in preventing the spread of disease.<sup>15</sup> The need to study and enforce preventive and control measures is highlighted to manage and prevent mastitis more effectively. The overall prevalence of mastitis was lower in buffaloes compared to crossbred cows.<sup>14</sup> This lower prevalence in buffaloes than cows might be attributed to the tighter teat sphincter of buffaloes, which could potentially reduce the entry of pathogens into the udder.<sup>16</sup> Results indicate that 86.2 % of the tested cows had SCM in one or more quarters. The quarter-wise prevalence of SCM can exhibit notable fluctuations throughout the year. Several factors contribute to these variations, including environmental conditions, cow physiology, and management practices. During the winter months, SCM prevalence tends to be elevated.<sup>17</sup> Cold weather and decreased ventilation in barns can create a favorable environment for mastitis-causing pathogens. Cows may also experience stress due to temperature fluctuations, which can weaken their immune systems and make them more susceptible to infections. Additionally, reduced outdoor access and increased confinement in winter can contribute to hygiene challenges in dairy operations. In spring, as temperatures rise and cows have access to fresh pasture, the prevalence of SCM may decrease. Improved hygiene and reduced stress levels due to better environmental conditions can contribute to lower infection rates during this quarter.<sup>18</sup>

**Table 3** Sub-clinical mastitis (SCM) in dairy cows

Quarter	Number of quarters screened	Number of quarters functioned	Absence of SCM (%)	Sore of CMT (%) (Number)	Sore of CMT (%) (Number) +	Sore of CMT (%) (Number) ++	Sore of CMT (%) (Number) +++	Total positive (%) (Number)	Blind Quarter (%) (Number)
LF	125	111	81.98	4.5	6.3	3.6	3.6	18.01	11.2
LH	125	109	77.06	10.09	5.5	4.58	2.75	22.93	12.8
RF	125	122	63.78	15.57	9.01	7.37	4.91	36.88	2.4
RH	125	107	71.02	9.34	13.07	2.88	3.77	28.97	14.4
Total	500	449	73.05	10.02	8.46	4.67	3.78	26.94	10.02

Summer can present a mixed picture. While warmer weather and increased ventilation may reduce the risk of SCM, the presence of flies and other vectors can contribute to higher transmission rates. Proper fly control measures are essential to mitigate this risk. The quarter-wise prevalence of subclinical mastitis in dairy cows exhibits distinct seasonal patterns. Understanding these variations is essential for effective mastitis management. By tailoring practices to seasonal conditions and implementing targeted strategies, dairy farmers can reduce the impact of SCM on milk quality and production, ultimately benefiting both the farm's profitability and animal welfare.<sup>19</sup>

<sup>20</sup>Reported that the right fore quarter had the highest prevalence of subclinical mastitis (28.40%), followed by the right hind quarter (24.29%), the left fore quarter (19.43%), and the left hind quarter (17.07%). Right side quarters had a higher prevalence of SCM (26.36%) compared to the left side quarters (18.26%), while fore quarters had a higher prevalence (23.94%) compared to the hind quarters (20.69%)<sup>21</sup>. Found that out of all the quarters, the right hind quarters had the highest prevalence (29.67%), followed by the left hind (26.37%), the left fore (23.07%), and the right fore (20.87%).<sup>22</sup>

Further research is needed to explore the complex interactions between seasons, cow physiology, and mastitis pathogens in greater detail.

### Relationship between CMT score with lactation length

Table 4 provides information about the distribution of California mastitis test (CMT) scores among dairy cows in different lactation stages, along with the corresponding milk yield values. CMT score; this represents the different levels of CMT scores, including + (single plus), ++ (double plus), and +++ (triple plus). Early lactation (0-90 days); this shows the distribution of cows in the early lactation stage based on their CMT scores. Mid lactation (90-180 days); this presents the distribution of cows in the mid lactation stage based on their CMT scores. Late lactation (>180 days); this indicates the distribution of cows in the late lactation stage based on their CMT scores. Total positive; these rows show the total number of cows that tested positive for subclinical mastitis (combination of +, ++, and +++ scores) in each lactation stage. Milk Yield (in Liters); this provides the corresponding average milk yield values for each lactation stage. In the early lactation stage (0-90 days), 29.54% of cows had a + CMT score, 18.18% had a ++ score, and 6.81% had a +++ score. In the mid lactation stage (90-180 days), 15.90% had a + CMT score, 11.36% had a ++ score, and 2.27% had a +++ score. In the late lactation stage (>180 days), 6.81% had a + CMT score, 9.09% had a ++ score, and no cows had a +++ score. The "Total Positive" rows provide the overall number of cows testing positive for subclinical mastitis in each lactation stage. Milk yield values decrease as lactation progresses, with the highest milk yield (2.5 liters) in early lactation, followed by 1.0 liter in mid lactation, and the lowest (0.75 liters) in late lactation. Subclinical mastitis (SCM) is also related to various factors such as age, parity,

and lactation length. Age and mastitis; subclinical mastitis tends to increase with age, which is consistent with findings from other studies such as.<sup>11,6, 20</sup> The fluctuation in prevalence might be influenced by the varying number of cows in different age groups. Older cows are more prone to exposure to mastitis pathogens due to increased exposure over time and changes in the udder's defense mechanisms as they age. Lactation length and sphincter potency indicate that longer lactation length might contribute to increased exposure to potential infection sources, influencing the prevalence of SCM. Early lactation stages are associated with the highest subclinical mastitis rates, possibly due to variations in teat sphincter potency.<sup>23</sup> Also, age and leukocyte function could play roles as younger cows tend to have better polymorph nuclear leukocyte function compared to multiparous cows. This information is based on studies of.<sup>5,8</sup> Parity and mastitis; subclinical mastitis is known to increase with parity advancement (the number of times a cow has calved). This finding aligns with results from previous studies of.<sup>11,19,20,24</sup> CMT and lactation stage; the CMT (California mastitis test) results in cows are correlated with the length of lactation. The study appears to have been conducted on cows in different lactation stages (early, mid, and late). Overall, SCM has shown specific associations with age, parity, lactation length, and other factors when studying the prevalence of subclinical mastitis. The relationship between CMT scores and lactation length is a complex and multifaceted one, influenced by various factors. High CMT Scores and Shortened Lactation Length, elevated CMT scores are often associated with intramammary infections, including subclinical mastitis. Cows with persistent or severe mastitis may experience a shortened lactation length due to decreased milk production, increased culling rates, or treatment-related issues. In such cases, high CMT scores could be indicative of lactation challenges and reduced productivity. Low CMT Scores and Extended Lactation Length, cows with consistently low CMT scores are less likely to suffer from mastitis-related productivity issues. This may lead to a longer lactation length, as these cows maintain higher milk production levels over time. However, it's important to note that other factors, such as reproductive performance and overall health, also play a significant role in determining lactation length. Management and Treatment, early detection, and management of mastitis through CMT testing can positively impact lactation length. Prompt treatment of infected cows can prevent severe cases and maintain milk production, potentially extending lactation. Individual Variability, cows vary in their susceptibility to mastitis and response to treatment. Some may maintain longer lactations despite occasional high CMT scores, while others may experience early drying off due to recurrent infections. The findings in the current study necessitate further deep study to provide a broader context in the subject. The relationship between CMT scores and lactation length in dairy cows is influenced by multiple factors, including the severity and persistence of mastitis, management practices, and individual cow characteristics. While elevated CMT scores can be indicative of mastitis-related challenges that may lead to shortened lactation lengths, they are not the sole determinant. Effective mastitis management, including

early detection and treatment through CMT testing, can positively impact lactation length by minimizing the impact of mastitis on milk production. Further research is needed to explore this relationship in greater detail, considering the complexity of factors influencing both CMT scores and lactation length.

**Table 4** Relationship between CMT score and the lactation phase

CMT Score	Early Lactation 0-90 (days)	Mid Lactation 90-180 (days)	Late Lactation >180 (days)
+	13 (29.54%)	7 (15.90%)	3 (6.81%)
++	8 (18.18%)	5 (11.36%)	4 (9.09%)
+++	3 (6.81%)	1 (2.27%)	0 (0%)
Total cows tested	38	38	38
Total Positive	24%	13%	7%
Milk Yield (in Liters)	2.5	1	0.75

The findings in the current study necessitate further deep studied to provide a broader context in the subject.

### Incidence of the disease

The incidence of mastitis varies with different seasons, with the highest reported cases during the monsoon season and the lowest during summer. Around 46% of affected animals experienced mastitis within a 30-90-day period after calving (lactation stage). This higher susceptibility in crossbred cows might be attributed to factors such as differences in teat anatomy. Challenges and prevention efforts; Developing vaccines for mastitis prevention has been attempted globally, but the disease's complexity involving various organisms presents a challenge. Mastitis detection and management such as routine mastitis detection tests are essential for identifying infected animals. Positive cases of subclinical mastitis should be isolated and treated promptly. Proper washing and hygiene practices are crucial for minimizing disease spread during milking.<sup>25</sup> Comfortable conditions for animals, dry bedding, and proper removal of dung and urine can contribute to preventing infections. Dry cow therapy with long-acting antibiotics at the end of lactation can help reduce new infections during the peri-parturient period (around calving). The incidence of these diseases related to subclinical mastitis may vary depending on the management practices employed by dairy farmers, the effectiveness of mastitis control programs, and the prevalence of mastitis-causing pathogens in the herd. Early detection and management of subclinical mastitis through methods like regular SCC testing and California Mastitis Test (CMT) can help reduce the incidence of these related diseases and improve overall herd health and productivity.

### Conclusion

A total of 460 quarters from two herds on an organized dairy farm were analyzed. Among these quarters, 132 quarters (28.69%) were found to be infected with mastitis. Among all the quarters analyzed, only one quarter of the udder was more affected in both herds. This suggests a localized distribution of mastitis within the udder. The prevalence of CMT score 1 (indicative of subclinical mastitis) in Herd 1 was 45%, while it was 25% in Herd 2. CMT scores provide insight into the prevalence of subclinical mastitis. CMT scores were found to increase in the early lactation stage, indicating a higher prevalence of mastitis during this phase of lactation. Infected cows with mastitis in the late lactation stage had an average milk yield of 3.6 liters. The study found that the level of intramammary infection in dairy cows was higher in the first and second parity. This increased prevalence

among first and second parity cows might be due to these animals being naive to environmental pathogens during their initial lactation. Overall, the study provides insights into the distribution of mastitis across udder quarters, the prevalence of subclinical mastitis based on CMT scores, the relationship between CMT scores and lactation stage, and the influence of parity on intramammary infection. These findings contribute to understanding mastitis prevalence patterns and potential risk factors on organized dairy farms.<sup>26</sup>

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### Conflicts of interest

Author declares there is no conflict of interest in publishing the article.

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