

Volume 4 Issue 8 August 2022

**Review Article** 

# On Farm Diagnostics and Preventive Measures for Mastitis in Dairy Bovines Category: Review Article

## Gayathri S Lal<sup>1</sup>\*, Mukesh Bhakat<sup>2</sup>, TK Mohanty<sup>2</sup> and S Maiti<sup>3</sup>

<sup>1</sup>PhD Research Scholar, LPM Division, ICAR-NDRI, Haryana, India <sup>2</sup>Principal Scientist, LPM Division, ICAR-NDRI, Haryana, India <sup>3</sup>Senior Scientist, Dairy Extension Division, ICAR-NDRI, Haryana, India **\*Corresponding Author**: Gayathri S Lal, PhD Research Scholar, LPM Division, ICAR-NDRI, Haryana, India. **DOI:** 10.31080/ASVS.2022.04.0482 Received: July 18, 2022 Published: July 25, 2022 © All rights are reserved by Gayathri S Lal., *et al.* 

## Abstract

Mastitis is defined as a multi-etiological disease that causes inflammation of the udder. It is one of the costliest production diseases accounting for an economic loss of 100 trillion US dollars globally and approximately Rs. 7165.51 crores in India. The incidence and prevalence rate is higher in sub-clinical mastitis than clinical mastitis in most herds throughout the World. It affects the quantity and quality of milk as well as the udder health of dairy animals. Poor sanitation and hygienic measures account for 67.8 percent of total factors responsible for flaring mastitic pathogens such as *E. coli, Staph. aureus* and *Strept. uberis*. Effective and early diagnosis of mastitis is highly essential in eradicating the disease from any herd. Along with the effective diagnosis, preventive measures also play a significant role in minimizing the cases of mastitis.

Keywords: Diagnosis; Incidence; Mastitis; Prevalence; Prevention

## Introduction

The simple definition of Mastitis is "inflammation of the mammary gland", especially of parenchyma cells. Several factors lead to mastitis, which manifests as the deviation in physical as well as chemical properties of milk and patho-physiological changes in the glandular tissues [1]. Mastitis is classified as clinical and sub-clinical mastitis. [2] point out that there is no visible change in the udder quarters or milk in the case of sub-clinical mastitis. The prevalence rate of sub-clinical mastitis in India ranges from 10-50 percent and that of clinical mastitis is 1-10 percent only. Production loss in terms of milk is 17.5 percent in the case of subclinical mastitis. The prevalence of sub-clinical mastitis is 15-40 times more than clinical mastitis, making the former more critical in any dairy farm management aspect. In addition, Sub-clinical mastitis detection is difficult [3] and is of long duration in nature. If unnoticed, the cases of sub-clinical mastitis may lead to prolonged economic loss and clinical mastitis. Around 65 percent of the mastitis cases are due to improper sanitation and hygienic practices followed among the dairy farms [4].

## **Classification of mastitis**

Mastitis is classified as environmental mastitis, contagious mastitis and gangrenous mastitis, based on the source of pathogen and their mode of transmission. The primary causative organism for environmental mastitis is E. coli, an opportunistic pathogen that directly invades the teat when cows expose themselves to the contaminated environment. The prevalence rate is less than 10 percent in any herd. The primary etiological agents for contagious mastitis are Staphylococcus aureus, Streptococcus dysgalactiae and Streptococcus agalactiae, which resides on teat skin. Inter mammary transmission occurs during milking. Sub-clinical mastitis, clinical mastitis, and chronic mastitis are the further subdivisions of contagious mastitis. The sub-clinical mastitis is characterized by the initial phase, a milder phase, during which no symptoms are manifested, followed by the mastitic phase where 10-20 percent reduction in milk yield is evident. If untreated, it may lead to clinical mastitis and other stages [2]. Clinical mastitis is characterized by cardinal

Citation: Gayathri S Lal., et al. "On Farm Diagnostics and Preventive Measures for Mastitis in Dairy Bovines Category: Review Article". Acta Scientific Veterinary Sciences 4.8 (2022): 158-169.

signs of inflammation and adversely affects early lactation and reproductive performance. In addition, clinical mastitis that causes severe mastitis is termed as per-acute mastitis. Reduction in milk yield, deviation in milk constituents accompanied by signs of fever (104-106 °F), depression, shivering, loss of appetite and loss of weight are the characteristics features of per-acute mastitis. Sometimes death is the sequelae in severe cases; however, incidences are generally very few. Another form is acute mastitis manifested as minor swelling in the infected quarter with flakes or clots in yellowish watery milk. Here no visible change of the udder is noticed. Chronic mastitis is a form of subclinical mastitis and may change to sub-acute or acute forms of mastitis. Chronic mastitis persists for months, continues from one to another lactation and may affect ovarian follicle development during later stages. The gangrenous mastitis isotherwise known as "blue bag." Here the udder gets cold, and within 3-4 days, the udder turns blue color, ultimately leading to the death of the animal. It is a severe form of mastitis, where the pathogens induce thrombosis leading to infarction and gangrene formation. Causative organisms are Mannheimia haemolytica and *Staphylococcus aureus* [6].

#### **Mastitis-etiology**

The major pathogens causing mastitis are Staphylococcus aureus, Streptococcus uberis, E. coli, Streptococcus bovis, Streptococcus agalactiae, Streptococcus dysgalactiae, and Klebsiella pneumonia. The minor pathogens like Proteus spp., Brucella abortus, Klebsiella oxytoca, Mycoplasma spp., Pseudomonas aeruginosa, Nocardia spp., Pasteurella spp., Prototheca zopfii, Corynebacterium bovis, Prototheca wickerhamii and yeast attributes to the transmission of mastitis from subclinical to next stage of mastitis. S. aureus beholds the major role for causing subclinical mastitis, accounting for 25.64 percent in specific mastitis, 12.24 percent in latent cases and owing to its adaptational talent in surviving extreme environments [6]. Throughout the World, several studies have been conducted regarding coagulase-negative staphylococci (CNS), the most isolated pathogen from milk and its prevalence rate is the highest in crossbred cattle. CNS is regarded as the rising mastitis pathogen, though they belong to normal skin microbiota. [5] reported that the CNS forms a protective biofilm, which enables them to linger on to the milking machine and milker's hand, which aids in the spread of infection. Unhygienic circumstances enhance the microbes' favorable condition to flare up as an opportunistic pathogen in the teat, causing mastitis. Another commonly encountered mastitic pathogen is Coryne bacteria and persists with routine teat dip usage but could be eliminated using dry cow antibiotic therapy. In New Zealand, the primary mastitis-causing organism is *Streptococcus uberis*, in the Midwestern United States, it is coliforms and in India, it is *Staphylococcus sp.*, *Streptococcus sp.*, and *E. coli* [6].

#### **Factors causing mastitis**

The major factors that affect the spreading of mastitis include the agro-climatic condition of the area, socio-cultural practices of the people, poor sanitation and hygienic practices adopted, nonavailability of veterinary services on-time and poor literacy awareness level among the farmers. Among them, poor sanitation and hygienic adapted accounts for 67.86 percent and which in turn causes the mastitis-causing pathogen to flare up [4] (Figure 1). Milking machine factors also contribute primarily to the occurrence of mastitis. Other factors associated with mastitis include age, breed, parity, stage of lactation, milking speed, udder and teat morphology. In crossbred cows, the chances of subclinical mastitis among the animals having a pendulous shape of the udder are the highest, followed by goat type, unbalanced, trough-shaped and round udder [7]. Small teat-sized animals are more prone to mastitis than medium or large teat-sized animals because a shorter teat canal enables the microbes to move upward without much hindrance in comparison to a large teat canal.

Similarly, pendulous-shaped udder of Sahiwal and Murrah buffaloes [8] are more prone to mastitis than other udder shapes [9]. In crossbred cows, conical teats are more prone to mastitis than other shapes such as cylindrical and bottle-shaped teats [10]. In addition, the first 3 weeks of the dry period and during the first month of lactation, the animals are more prone to mastitis.

#### Economic loss due to mastitis

India beholds the largest dairy farming community that accounts for around 13.90 million farmers. These farmers' economic and production loss are not small enough, but it is worth addressing enough. The production aspects are affected, and mastitis reduces the reproduction efficiency. Several predisposing factors of mastitis include inadequate sanitation, hygienic practices at the herd level, and mastitis has increased the burden of economic loss among the farmers. In addition, antimicrobial residues in milk contribute to the huge loss of milk concerning the public health safety issues [11]. Culling of the animals affected with episodes of mastitis at herd level incur a severe loss to the farmers. [4] reported that the anticipated expenditure of 49 percent accounts for milk

Citation: Gayathri S Lal., et al. "On Farm Diagnostics and Preventive Measures for Mastitis in Dairy Bovines Category: Review Article". Acta Scientific Veterinary Sciences 4.8 (2022): 158-169.



Figure 1: Factors causing the spread of mastitic pathogens and factors causing the teat end trauma due to machine milking [4,2].

quality and 37 percent for veterinary expenses, totally the loss incurred per lactation is Rs. 1390 for crossbred cows in India. For the US dairy industry, the economic loss due to mastitis accounts for \$2 billion [12] and a case of clinical mastitis in a herd account for \$95 to \$211[13]. The total economic loss incurred due to mastitis by Purvanchal and Uttar Pradesh farmers was INR 5, 210 in nondescript cattle, INR 36, 795 in crossbred cattle and INR 24, 175 in buffalo [14]. In addition, the economic loss per animal per lactation was Rs.868, Rs.1314 and Rs.1272 in the case of non-descript cattle, crossbred cattle and buffaloes, respectively [14]. Similarly, the farmers of Hyderabad suffered a loss of Rs.326 per animal. Correspondingly, the average loss per month in optimistic and pessimistic scenarios of the Indian condition is Rs. 3206.55, Rs. 2119.67 and Rs.1708.89 as well as Rs. 3549.59, Rs. 2448.03 and Rs. 1934.78, respectively, for crossbred cows, indigenous cows and buffaloes. The average treatment cost for mastitis per animal accounts for Rs. 525, Rs. 695.53 and Rs. 647.36 for crossbred cows, indigenous cows and buffaloes, respectively in Indian condition. The production loss accounts for 39.53% (Rs. 316.67), 47.07% (Rs. 618.56), and 49.12% (Rs. 625) for non-descript cows, crossbred cows, and buffaloes, respectively [15,16] reported that mastitis indirectly causes an increase in the number of deaths among human beings, which is due to the proliferation of antibiotic-resistant superbugs. These superbugs are generated due to the usage of antibiotics for the treatment of mastitis. Resistant superbugs lead to the death of 700000 people per year and are estimated to reach 10 million by 2050. The economic loss of 100 trillion US dollars across the World is solely due to mastitis.

## Prevalence and incidence rate of clinical and subclinical mastitis

The incidence and prevalence of mastitis are increasing day by day, along with the increasing milk productivity of dairy animals. The chance of clinical mastitis increases with increasing parity and adversely affects the milk quality. Sub-clinical mastitis is 15-40 times more prevalent among the dairy herds than clinical mastitis and produces a definitive impact among the bovine mastitis. In general, the prevalence of mastitis among the non-descript cattle is comparatively less to that of the crossbred animals in India. According to meta-analysis and systematic review study for the duration of 1967-2019, [17] revealed that the pooled prevalence of subclinical mastitis and clinical mastitis was 42 and 15 percent in the World and 45 and 18 percent in India, respectively. The prevalence rate was higher in North America for sub-clinical mastitis and clinical mastitis; Europe is in the continent-wise analysis. In addition, at the country level, higher subclinical mastitis prevalence was observed in Uganda and clinical mastitis in the United Kingdom [17]. Several other studies reported that the prevalence of mastitis due to CNS was highest among the countries like Finland -50% and Canada -51% and for subclinical mastitis caused due to CNS; it is

Citation: Gayathri S Lal., et al. "On Farm Diagnostics and Preventive Measures for Mastitis in Dairy Bovines Category: Review Article". Acta Scientific Veterinary Sciences 4.8 (2022): 158-169.





Figure 2: The prevalence and incidence rate of clinical mastitis among the different countries across the World [49,50].

13.7% and 16.6% [18], respectively. The prevalence and incidence rate of clinical mastitis in several countries has been depicted in figure 2.

disease and need effective early diagnostic mechanism and preventive strategy to reduce the prevalence rate. The prevalence rate of clinical mastitis and sub-clinical mastitis ranges from 5.5-37.09 percent (Figure 3) and 27.37- 60.25 percent (Figure 3), respectively, among the different states of India.

The prevalence of mastitis among the states of India ranges from 25.63 to 97.61%, indicating that mastitis is widely prevalent



Figure 3: Prevalence rate of clinical mastitis among different states of India [19,21,25].

[3] reported that the overall prevalence rate of mastitis, subclinical mastitis and clinical mastitis was 31.21, 25.63 and 5.58 percent, respectively, among the dairy cows in Parbhani, Maharashtra. In addition, he reported that HF crossbred cows (39.01%) showed more prevalence than the native (29.52%) cows. Other studies reported that the prevalence of mastitis is around 61.9 percent in Jersey crossbred [19] and 31.75 percent in Holstein crossbred and it is higher in comparison to the Zebu cattle [20]. [21] reported the incidence rate of mastitis in HF crossbred (31.94 -50.47 percent), Brown Swiss crossbred (20.00 - 50.00 percent), Sahiwal (25.60 - 47.83 percent), Tharparkar cows (18.18 - 50.00 percent) and Murrah buffaloes (17.98 -37.50 percent) based on data of 2000-2011 under organized herd. [17] reported that world buffaloes showed a higher prevalence rate than the world cattle population. The prevalence rate of mastitis identified in terms of somatic cell count was more among HF and Brown Swiss crosses high producing cows [22]. The prevalence of subclinical mastitis in crossbred cows indifferent parts of the country was reported at 57.80% [23]; 50.81% [20] and 62.6% [24], respectively.



Figure 4: Prevalence rate of sub-clinical mastitis among different states of India.

[25] reported that, in Uttar Pradesh, the prevalence rate of subclinical mastitis during July - August among cattle is comparatively higher and accounts for 42.93 percent than clinical mastitis (15.18 percent). [19] reported that the prevalence of mastitis among heifers during autumn, summer, winter and spring is 6.35, 15.87, 34.92 and 42.86 percent, respectively. The prevalence of mastitis among the age group of cattle showed a pattern, adult (39.44%) > young (31.66%) > old (21.43%), in stage of lactation, early (35.25%), > mid (29.69%) > late (18.82%) and in parity, first - third (26.36%) > fourth - sixth (51.61%) > 7 or above (16.28%) [3].The prevalence of mastitis among udder quarters were in the pattern of LH (36.04%) > RH (29.95%) > LF (20.81%) > RF (15.99%). The prevalence of quarters-wise subclinical mastitis in dairy cows in organized farms of India was reported 23.25% [26] and 23% [27]. Udder shape is a critical trait for the selection of dairy animals, and it is also associated with the incidence of mastitis, which is an unavoidable factor requiring more scientific attention. [7] reported a higher incidence rate of subclinical mastitis in the pendulous shaped udder (93.54 percent) followed by unbalanced (41.66 percent), goat type (33.33 percent), round (26.31 percent) and trough-shaped udders (10.95 percent) in crossbred cows. In concomitant to the above study, [9] reported that pendulous shaped udder showed the highest incidence rate of clinical and subclinical mastitis in Sahiwal cows and Murrah buffaloes (88.89 and 71.43 percent, respectively) followed by goaty (83.33 and 40.00 percent), round (38.46 and 18.18 percent) and trough (31.43 and 9.52 percent) shaped udder. In HF crossbred, he observed the highest incidence rate in goaty (77.78 percent) followed by pendulous

Citation: Gayathri S Lal., et al. "On Farm Diagnostics and Preventive Measures for Mastitis in Dairy Bovines Category: Review Article". Acta Scientific Veterinary Sciences 4.8 (2022): 158-169.

(75.00 percent), round (36.84 percent), and trough (21.88 percent) shaped udder. [28] observed a higher incidence of mastitis among bowl (35.24%) shaped udder followed by round (27.69%) and cup (6.88%) shaped udder of indigenous cattle. It has also been observed that smaller teat dairy animals are more prone to mastitis than their counterparts having medium and larger teat as the upward movement of microbes is faster, without much hindrance in shorter teat canal than in comparison to large teat canal. Thus, all these above studies reflect that udder morphology plays a significant role in the incidence of mastitis among dairy herds.

#### Milking environment and mastitis

The milking environment plays a vital role in the causation and transmission of mastitis among dairy animals in a herd and is a common reason for mastitis. Environmental mastitis is portraved as a disease syndrome rather than as a single disease as it has several causative agents and many contributing causes at the host and environmental levels. The major culprits for environmental mastitis are Staphylococcus aureus, Streptococcus uberis and other streptococcal species, including Streptococcus agalactiae. The major source of these pathogens, especially Escherichia coli, S. uberis includes piled up bedding, dung, and the indoor environment of the housing system of dairy cattle. The clinical signs manifested by each causative microbe vary. Coliform mastitis is usually manifested as moderate (asymptomatic, abnormalities in milk and mammary gland), mild (only milk abnormalities) or persistently subclinical (asymptomatic). In addition, it is transient in nature and the disease effect depends on host factors such as lactation stage, energy balance, vitamin deficiency and vaccination status [29]. S. *agalactiae* causes mild-to-moderate forms of clinical mastitis [30]. Organisms such as Klebsiellapneumoniae and S. agalactiae propagate via the faeco-oral route. Assessment of somatic cell count during the early stages of the dry period and treating the positive animals during the dry period can reduce coliform mastitis during the early stages of lactation. Similarly, mastitis caused due to Klebsiella, Citrobacter, and Serratia spp. could also be controlled early. In addition, the mortality caused due to Klebsiella and the bacteremia associated with mastitis is also reported in dairy farms. The teat end microbiota and milking speed [31], especially in US dairy farms, play a crucial role towards the major pathogens causing environmental mastitis. To prevent environmental mastitis, it has to resolve whether clinical mastitis manifested during early lactation is due to infections during the dry period or lactation. Thus,

proper management and practice of hygienic protocols can reduce the spread of environmental mastitis, which further prevents the usage of antibiotics. Vaccinations against coliforms are successfully practiced among some dairy farms as a preventive measure, but not all mastitis-causing organisms have vaccines. Early diagnosis and identification of the disease using advanced technological tools form a key in controlling the spread of environmental mastitis. The creation of awareness among the dairy farmers about the standard management practices via government initiatives is also effective in tackling environmental mastitis.

## **Mastitis diagnosis**

Mastitis describes its effect in the form of chemical, physical, and bacteriological changes in milk and pathological changes in glandular tissue [32]. Mastitis causes economic and production loss that needs tackling through early assessment via routine diagnostic and prognostic procedures. The following routine diagnostic tests practiced throughout the World for mastitis include:

- Physical examination of the udder assesses the teat and udder shape, contour, consistency, and size immediately after milking [33], which aids in successfully detecting mastitis.
- Strip cup test is commonly used in milking parlours for detecting clinical mastitis. Foremilk squirting to the strip reveals blood, flakes, clots and wateriness of milk, indicating mastitis.
- California mastitis test is an eeasy, cheap, and rapid screening test, which qualitatively estimates an increase in somatic cells in the milk during mastitis. Usually, the milk samples are scored as 0, 1, 2, 3 depending upon gel formation. The formation of more gel indicates higher somatic cell count and more infection levels.
- Wisconsin mastitis test, a laboratory test conducted primarily on milk samples from bulk tank. It is similar to CMT and is used to forecast the average number of somatic cells present in the milk.
- Modified white side test detects the increased leukocytic count in the milk. Normal milk produce flakes with the test but mastitic milk form an opaque fluid.
- pH Determination test measures the pH of the milk sample. During the late lactation and dry period, mastitis produces alkaline milk, which can be detected using the test.

Citation: Gayathri S Lal., et al. "On Farm Diagnostics and Preventive Measures for Mastitis in Dairy Bovines Category: Review Article". Acta Scientific Veterinary Sciences 4.8 (2022): 158-169.

- Chloride test detects the increased amount of chloride in the mastitic milk.
- Electrical conductivity test is a physical method to detect mastitis. The test detects the electrical conductivity of the given sample of milk with a unit of milk milli Siemens per centimeter (mS/cm). An electrical conductivity meter (milk checker or digital mastitis detector) is used for the test; EC level increases in mastitis due to an increase in Na<sup>+</sup> and Cl<sup>-</sup> concentration in the milk.
- Somatic cell count of milk is of three types direct microscopic somatic cell count (DMSCC), the bulk milk somatic cell count (BMSCC) and individual cow somatic cell count (ICSCC)). [32] reported that the somatic cell count more than 250000/ml were considered to be indicative of inflammation; whereas counts less than 100,000/ml was indicative of normal udder and counts more than 500,000 cells/ml was indicative of infection.
- N-acetyl-β-D-glucosaminidase test (NAGASE) measures the cell-associated enzyme N-acetyl-β-D-glucosaminidase in milk. The activity ranges of NAGASE for normal milk (< 0.5 x 10<sup>4</sup> cells/ml) and mastitic milk (1.5 x 10<sup>4</sup> cells/ml) are 0.0053 and 0.034/mole/min/ml, respectively.
- Methylene blue reduction test (MBRT) estimates the chemical reaction of the respiratory activity of bacteria in milk. When the bacteria consume all oxygen in milk, the blue color turns white.
- Milk anti trypsin assay (MAUM TEST) estimates milk antitrypsin activity due to leakage of blood á-1 Protease inhibitor into milk and represents increased permeability. Normal milk contains ≤ 200 BEN units/ml of trypsin inhibitor. During mastitis, the level of trypsin inhibitor in milk increases.

Apart from the above methods, simple, non-invasive prognostic tools are the need of the hour. Rapid, non-invasive and cow side diagnostic tool provides prospective use in the field-level condition, which is essential in assessing udder health. In such circumstances, early assessment of mastitis can bring a lot of difference and Infrared thermography (IRT) can be a promising tool. Researchers have used IRT as a tool to access the udder skin surface temperature (USST) changes in healthy and mastitis-affected quarters of dairy animals. Using IRT, subclinical [34] and clinical mastitis [9,35] can be identified using the change in USST and can further be confirmed using CMT and SCC.

#### California mastitis test (CMT) test as mastitis diagnostic tool

California mastitis test (CMT) is one of the most frequently used diagnostic tools for mastitis. The test estimates the number of somatic cells present in milk. Around 75 percent of the somatic cells are leucocytes, and the rest are epithelial cells. Leucocytes get increased during mastitis. CMT test is carried out by mixing (10 sec) the test reagent (CMT reagent) with an equal quantity of milk (around 3ml) in a marked CMT paddle cup to identify the quarters from which milk is collected. The reagent reacts with the somatic cell's DNA in the milk to form a gel. The reaction is visually scored as 0, T (Trace), 1, 2, or 3, depending upon the quality and quantity of gel formation. The formation of more gel indicates a higher somatic cell count from which we can differentiate it as sub-clinical mastitis or clinical mastitis.

The fundamental behind the California mastitis test is that the test qualitatively estimates the amount of DNA in milk secretions. There is a direct correlation between concentrations of DNA as well as white blood cells in the milk. The reagent lyses the cells and forms gels. The extent of gel formation depends on the number of leucocytes in milk. CMT scores are usually higher in fresh cow milk and towards the end of lactation and need not be mistaken as sub-clinical mastitis. In addition, there is a positive correlation between the scores of CMT and the values of SCC.

### **Correlation between CMT scores and SCC**

The correlation between CMT and SCC has been depicted in the table below [36, 37] conducted a study and analyzed 601 milk samples from 151 cows using the Modified California Mastitis Test to identify subclinical mastitis. He further classified the positive 200 samples into three groups such as + (weakly positive-13.81percentage), ++ (distinctively positive-11.48 percentage) and +++ (strongly positive- 7.99 percentage). The overall prevalence of subclinical mastitis based on the Modified California Mastitis Test was 70.19% (animal-wise) and 33.27% (Quarter-wise). [38] tested 1978 cows from 2150 cows by CMT and observed 56.02% (1108 cows) prevalence. [39] reported subclinical mastitis prevalence in quarter wise 8.12, 22.88 and 69.00 per cent positive for CMT (+), CMT (++) and CMT (+++), respectively. Similarly, [40] evaluated

Citation: Gayathri S Lal., et al. "On Farm Diagnostics and Preventive Measures for Mastitis in Dairy Bovines Category: Review Article". Acta Scientific Veterinary Sciences 4.8 (2022): 158-169.

CMT Score	Score Somatic Cell Range	
N	0 to 200,000.	
1	200,000 to 400,000.	
2	400,000 to 1,200,000.	
3	Over 5,000,000	

#### Table a

sub-clinical mastitis using CMT and found out that 258 (66.85%), 85 (22.02%) and 43 (11.13%) milk samples as CMT (+), CMT (++) and CMT (+++). Thus, CMT score is one of the diagnostic tools, which can be used along with SCC.

## Somatic cell counts (SCC) as mastitis diagnostic tool

Somatic Cell Counts of Milk (SCC) is an effective tool in detecting mastitis throughout the World. In this test, the number of somatic cells is counted. There are several standards worldwide regarding the acceptable level of SCC in milk. National Dairy Research Institute has put forth a standard of SCC in the milk of indigenous cattle-1-1.5 lakh/ml and buffalo up to one lakh/ml milk to monitor the prevalence of subclinical mastitis [41]. The table interprets the udder status according to the gold standard tests for mastitis-CMT and SCC [42].

CMT score	Visible reaction	Somatic cell count	Interpretation	
N (Negative)		0-200,000	Healthy quarter	
	Milk fluid and normal, no thickening	(0-25% of neutrophils)		
T (Trace)	Slight thickening, reaction, disappears in	200,000-400,000	Early subclinical mastitis	
	10 seconds	(30-40% of neutrophils)		
1 (Weak +ve)		400,000-1,200,000	Subclinical mastitis	
	Distinct thickening, no gel formation	(40-60% of neutrophils)		
2 (Distinct +ve)	Thickens immediately, begins to gel, levels	1,200,000-5,000,000	Clinical mastitis	
	in the bottom of cup	(60-70% of neutrophils)		
3 (Strong +ve)	Gel is formed, surface elevates, with a	Over 5,000,000 (70-80% of	Severe clinical mastitis	
	central peak above the mass	neutrophils)		

#### Table b

In DMSCC (direct microscopic somatic cell count), a thick smear of milk is made on a sterile glass slide and stains it using 1 percent methylene blue. Once the staining is complete, count 60 fields under a microscope [43]. Then calculate the number of cells per ml of milk using the formulae. Electronic somatic cell counters are currently available but require expertise and skill. Several factors influence the SCC in milk, such as productivity of the animal, health, lactation stage, parity, breed and environmental conditions, especially stressful period and hygienic practices adopted in the farm, *etc.* [22].

Excessive secretion of somatic cells in milk occurs at the time of infectionor any assault to udder. These cells act as the defense system and protect the mammary gland from external pathogens to a limit. In most developed countries, milk SCC is used as the marker [44] to assess the prevalence of mastitis in their farms. SCC is used

as the quality benchmark in some of the counties, such as New Zealand, Switzerland, China, Canada and the European Union, and the BMSCC limits are  $3-4 \times 10^5$  cells/mL, in South Africa and Brazil,  $5 \times 10^5$  cells/mL; and in the USA,  $7.5 \times 10^5$  cells/ml. However, in India, milk fat and SNF are still used to measure the quality of milk. Studies reported that the hand-milked milk of cows has more SCC than machine milked milk. Precision farming systems have got automatic detectors of SCC during milking. BMSCC (bulk milk somatic cell count) comparison was made 24 months before installing the automatic milking system until 48 months post-installation, and it was observed that BMSCC levels were significantly higher during 12-month post-installation. However, these decreased over time and even showed a significantly lower BMSCC after 36 months post-installation. These reports indicated that the initialization of milking machines in the farm increased SCC for initial days; later on, once the animal was adapted, the SCC count decreased.

Citation: Gayathri S Lal., et al. "On Farm Diagnostics and Preventive Measures for Mastitis in Dairy Bovines Category: Review Article". Acta Scientific Veterinary Sciences 4.8 (2022): 158-169.

Similarly, in the case of buffalo milk also, SCC is a clear indicator of mastitis. Studies regarding Buffalo milk SCC were conducted in various countries [45]. The buffalo milk SCC changes during parturition and involution and gets normal after two weeks. SCC of buffalo milk increased significantly higher around parturition and became normal at 14-days postpartum. Therefore, the chances of udder infection during the dry period are low, but if infection lodges during the dry period, then the chances of mastitis occurring are more in ensuing lactation. Another striking difference is that chances of SCC increase are low for buffaloes compared to cows. Day-to-day variation in SCC count for Murrah buffaloes milk has also been observed [46]. Detailed study regarding SCC helps in the analysis of immune status and buffaloes with high SCC portray high alkaline phosphates activity.

Flow cytometry is another tool to measure the SCC in milk. In order to make the somatic cell count uniform all over the World, International Dairy Federation and International Committee for Animal Recording launched a new project, which pertains to setting up an international reference system for SCC in raw milk. Even the Quick SCC app for iPhonesis in use. It allows real-time images of actual milk samples directly in the app and readily available reports at the laboratory level.

#### **Reduction of milk SCC at farm levels**

Several studies reported a strong association between dairy farm management and SCC [47]. Thus, measuring the SCC indicates the effectiveness of farm management. Effective hygienic practices carried out by milkers such as wearing protective coverings, selfhygiene measures, and effective pre- dips and post-dip can reduce the SCC in milk. Proper cleaning and disinfection of automated milking systems can also reduce SCC in milk. Regular inspection by supervisors is essential to ensure the proper functioning of the farm. If there are diseased animals, they should be milked towards the end, and the dairy animals should not be allowed to lie down immediately after milking, as the teat canal is open for 1-2 hours post milking. Loose housing system, cleaning the calving pen after each calving, quality bedding provision, dry cow udder monitoring for mastitis, use of blanket dry cow therapy for high yielding animals, micronutrient supplementation, management of udder hair, and frequent testing by CMTreduces SCC. In heifer, the chances of elevated SCC are there during the peripartum period. Assessment of milk's pH regularly aids in the control of mastitis. Citrate acts as the most effective buffer that regulates Ca2+ and H+ in the udder

and maintains normal pH. [14] reported that the administration of trisodium citrate @ 30 gm dissolved 50 ml of drinking water as a drench daily for 3-5 days or as the recovery is achieved is the best protocol for treating mastitis in buffaloes. During the treatment with tri-Sodium citrate the pH returns to normal level (~6.50) and removes the mastitis causing organism from udder. Intravenous administration of tri-Sodium citrate as 5% in normal saline is standardized for treatment of mastitis. The 50ml I/V doses of 5% solution of tri-Sodium citrate in sterilized normal saline given morning and evening cures the clinical cases in 1-3 days. Reports showed a strong relationship between udder health and mineral supplementation and teat dipping [48]. Thus, practices such as clean animal surroundings, regular monitoring and screening of animals using CMT/SCC, feeding antioxidants, prompt treatment of mastitis affected cows as well as culling of chronically mastitis affected cows, dipping practices, awareness about proper dry cow therapy and selection of animals against mastitis to improve SCC count in a herd. Henceforth, sound farm management practices and effectiveness in practicing the measures form the key to control SCC and mastitis.

### Conclusions

The economic loss due to mastitis is profound among both developed and developing countries. The prevalence rate and incidence rate of mastitis effectively signify its address with minimum delay. Diagnosis of mastitis is accurate in CMT and SCC evaluation of milk at the animal's level. Prevention of mastitis using effective control programmes needs further strengthening. Clean milk production protocols including dry cow therapy and management of dry animals needs further strengthening. Regular screening of animals in a herd, prompt treatment of mastitis-affected animals and improving the hygienic milk production management practices are the need of the hour. Usage of platform test such as CMT, WST and other tests need to be done at farmers level as early detection of mastitis is more important in containing its incidence. Overall assessment of the incidence of mastitis in the farm level must be done and regular culling of the chronically affected animals must be practiced. Above all, effective transfer of SCC management knowledge from the scientific community to the farmers' doorstep is essential to reduce the farmer's economic loss. Mastitis is a global concern and needs an effective tackling using non-invasive, simple, effective tool techniques such as IRT, which requires further validation and needs familiarization among the dairy farmers about the advancements in their field.

Citation: Gayathri S Lal., et al. "On Farm Diagnostics and Preventive Measures for Mastitis in Dairy Bovines Category: Review Article". Acta Scientific Veterinary Sciences 4.8 (2022): 158-169.

## **Bibliography**

- Constable PD., *et al.* "Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs, and Goats". Elsevier, USA 11.2 (2017): 46-84.
- Sharma N., *et al.* "Bovine Mastitis". 1<sup>st</sup> Edition, Satish Serial Publishing House Delhi, India (2012).
- 3. Shaikh SR., *et al.* "Epidemiological studies of mastitis in cows reared under different managemental system in and around Parbhani". *The Pharma Innovation Journal* 8.2 (2018): 01-05.
- Sinha MK., *et al.* "Subclinical Mastitis in Dairy Animals: Incidence, Economics, and Predisposing Factors". *The Scientific World Journal* 523984 (2014):1-4.
- 5. Pilipčincová I., *et al.* "Genotypic characterisation, of coagulase-negative *Stahpylococci* isolated from sheep milk in Slovakia". *Acta Veterinaria Brunensis* 79 (2010): 269-275.
- Krishnamoorthy P., et al. "Meta-analysis of Prevalence of Subclinical and Clinical Mastitis, Major Mastitis Pathogens in Dairy Cattle in India". *International Journal of Current Microbiology and Applied Sciences* 6.3 (2017): 1214-1234.
- Kamboj ML., *et al.* "Effect of udder and teat characteristics on incidences of subclinical mastitis in crossbred cows". *Indian Veterinary Journal* 85.8 (2008): 846-848.
- Prasad RMV., et al. "Studies on the udder and teat morphology and their relationship with milk yield in Murrah buffaloes". Livestock Research for Rural Development 22 (2010): 1-15.
- Danish Z., *et al.* "Udder and teat morphology and their relation with incidence of sub-clinical and clinical mastitis in Sahiwal, Karan Fries cows and Murrah buffaloes". *Journal of Entomology and Zoology Studies* 6.5 (2018): 2138-2141.
- Kamboj ML., *et al.* "Effect of udder and teat shapes and their measurements on somatic cell counts in milk of Karan-Fries cows". *Indian Journal of Dairy Science* 60.6 (2007): 435-440.
- Nielsen C. "Economic impact of mastitis in dairy cows. Doctoral Thesis". *Swedish University of Agricultural Sciences*, Uppsala, Sweden (2009).

- 12. Bewley JM. "Southeast Quality Milk Initiative: Implementing science-based recommendations to control mastitis and improve milk quality in the Southeast". In Proceedings. National Mastitis Council. USDA (2014).
- 13. Liang LM., *et al.* "Estimating US dairy clinical disease costs with a stochastic simulation model". *Journal of Dairy Sciences*, 100 (2017): 1472-1486.
- Singh D., *et al.* "Economic losses due to important diseases of bovines in central India". *Veterinary World* 7.8 (2014): 579-585.
- Bardhan D. "Economic losses due to clinical mastitis in organized dairy farms". *Indian Journal of Dairy Science* 66.2 (2013): 168-172.
- Blum B. "A biological alternative to antibiotics for dairy cows". Israel 21C. Uncover Israel. Weekly newsletter (2021).
- 17. Krishnamoorthy P, *et al.* "Global and countrywide prevalence of subclinical and clinical mastitis in dairy cattle and buffaloes by systematic review and meta-analysis". *Research in Veterinary Science* 136 (2021): 561-586.
- 18. Jakeen K El-Jakeea., *et al.* "Emerging of coagulase-negative staphylococci as a cause of mastitis in dairy animals: An environmental hazard". *International Journal of Veterinary Science and Medicine* 1.2 (2013): 1-12.
- 19. Tufani NA., *et al.* "Epidemiology and therapeutic management of bovine mastitis". *Indian Journal of Animal Research* 46.2 (2012): 148-151.
- 20. Awale MM., *et al.* "Bovine mastitis: a threat to economy". *Scientific Reports* 1 (2012): 295-305.
- 21. Jingar SC., *et al.* "Effect of Year Wise on Incidence of Mastitis in Crossbred, Indigenous Cattle and Murrah Buffaloes". *International Journal of Current Microbiology and Applied Sciences* 6.6 (2017): 2725-2728.
- Alhussien MN and Dang AK. "Milk somatic cells, factors influencing their release, future prospects, and practical utility in dairy animals: An overview". *Veterinary World* 11.5 (2018): 562-577.

Citation: Gayathri S Lal., et al. "On Farm Diagnostics and Preventive Measures for Mastitis in Dairy Bovines Category: Review Article". Acta Scientific Veterinary Sciences 4.8 (2022): 158-169.

- 23. Kumar V. "Studies on milk quality assessment with special reference to cow udder health and milking management, M. V. Sc thesis". Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana (2012).
- Abebe R., *et al.* "Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia". *BMC Veterinary Research* 12.1 (2016): 270-282.
- 25. De UK and Mukherjee R. "Prevalence of mastitis in cross bred cows". *Indian Veterinary Journal* 86 (2009): 858-859.
- Bangar YC., *et al.* "A systematic review and meta-analysis of prevalence of subclinical mastitis in dairy cows in India". *Tropical Animal Health Production* 47.2 (2015): 291-297.
- 27. Patel YG and Trivedi MM. "Quarter-Wise Prevalence of Subclinical Mastitis in Crossbred Cows". *Trends in Biosciences* 8 (2015): 4727-4729.
- Hussain R., *et al.* "Changes in some biochemical parameters and somatic cell counts in the milk of buffalo and cattle suffering from mastitis". *Pakistan Veterinary Journal* 32.3 (2012): 418-421.
- 29. Bradley AJ., *et al.* "An investigation of the dynamics of intramammary infections acquired during the dry period on European dairy farms". *Journal of Dairy Science* 98 (2015): 6029-6047.
- Cortinhas CS., *et al.* "Randomized clinical trial comparing ceftiofur hydrochloride with a positive control protocol for intramammary treatment of nonsevere clinical mastitis in dairy cows". *Journal of Dairy Science* 99 (2016): 5619-5628.
- Falentin H., *et al.* "Bovine teat microbiome analysis revealed reduced alpha diversity and significant changes in taxonomic profiles in quarters with a history of mastitis". *Frontiers in Microbiology* 7 (2016): 480-495.
- Radostits OM., *et al.* "Mastitis control in dairy herds". Herd Health Food Animal Production Medicine, 2<sup>nd</sup> edition. W. B. saunders, Philadelphia (1994): 229-273.
- Varshney JP and Mukherjee R. "Recent advances in management of bovine mastitis". *Intas Polivet* 3.1 (2002): 62-65.
- Silva Rodes Angelo Batista da., *et al.* "Exploratory data inference for detecting mastitis in dairy cattle". Acta Scientiarum". *Animal Sciences* 42 (2020): 1-12.

- 35. Sathiyabarathi M., *et al.* "Infrared thermography to monitor body and udder skin surface temperature differences in relation to subclinical and clinical mastitis condition in Karan Fries (Bos taurus × Bos indicus) crossbred cows". *Indian Journal of Animal Sciences* 88.6 (2018): 694-699.
- 36. Sargeant JM., *et al.* "Sensitivity and specificity of somatic cell count and California mastitis test for identifying intramammary infection in early lactation". *Journal of Dairy Science* 84 (2001): 2018–24.
- Lahamge MS., et al. "Prevalence of Subclinical Mastitis in Cows: In and Around Nagpur Region". International Journal of Current Microbiology and Applied Sciences 8.12 (2019): 83-88.
- Varatanovic N., *et al.* "Research on subclinical mastitis and its ethiological in different breeds of cow". *Biotechnology in Animal Husbandry* 31.3 (2015): 365-374.
- Risvanli A and Kalkan C. "The effect of age and breed on somatic cell count and microbiological isolation rates in milk of dairy cows with subclinical mastitis. Yuzuncu Yil Universitesi". *Veteriner Fakultesi Dergisi* 13 (2002): 84-87.
- Kasikci G., *et al.* "Relations between electrical conductivity, somatic cell count, California mastitis test and some quality parameters in the diagnosis of subclinical mastitis in dairy cows". *Turkish Journal of Veterinary and Animal Sciences* 36.1 (2012): 49-55.
- 41. Arora P. "NDRI 'sets' somatic cell counts limit in milk-Says it will reduce antibiotic residue, produce grade-A milk". *The Tribune*. Tribune News Service, Karnal (2020).
- 42. Mohanty AK., *et al.* "Mastitis in dairy animal: current concepts and future concerns". Satish Serial Publishing House, Delhi, India (2015): 1–5.
- 43. Castro A., *et al.* "Long-term variability of bulk milk somatic cell and bacterial counts associated with dairy farms moving from conventional to automatic milking systems". *Italian Journal of Animal Science* 17 (2017): 218-225.
- 44. Petzer IM., *et al.* "Somatic cell count thresholds in composite and quarter milk samples as indicator of bovine intramammary infection status". *Onderstepoort Journal of Veterinary Research* 84 (2017): 1269- 1284.

Citation: Gayathri S Lal., et al. "On Farm Diagnostics and Preventive Measures for Mastitis in Dairy Bovines Category: Review Article". Acta Scientific Veterinary Sciences 4.8 (2022): 158-169.

- 45. Sales DC., *et al.* "Relationship between the yield of mozzarella cheese of buffalo's milk, milk quality and the recovery of constituents in whey". *Journal of Animal Science* 94 (2016): 246-254.
- 46. Bombade K., *et al.* "Diurnal variation of milk somatic and differential leukocyte counts of Murrah buffaloes as influenced by different milk fractions, seasons and parities". *Biological Rhythm Research* 49 (2017): 151-163.
- Vissio C., *et al.* "Milking machine and udder health management factors associated with bulk milk somatic cell count in Uruguayan herds". *Preventive Veterinary Medicine* 150 (2018): 110-116.
- Emanuelson U and Nielsen C. "Weak associations between mastitis control measures and bulk milk somatic cell counts in Swedish dairy herds". *Journal of Dairy Science* 100 (2017): 6572-6576.
- 49. Amer S., *et al.* "Prevalence and etiology of mastitis in dairy cattle in El Oro Province, Ecuador". *Journal. Veterinary Medicine and Science* 80.6 (2018): 861-868.
- 50. Gao J., *et al.* "Incidence of clinical mastitis and distribution of pathogens on large Chinese dairy farms". *Journal of Dairy Science* 100 (2017): 4797-4806.

Citation: Gayathri S Lal., et al. "On Farm Diagnostics and Preventive Measures for Mastitis in Dairy Bovines Category: Review Article". Acta Scientific Veterinary Sciences 4.8 (2022): 158-169.