Review Article

The Window of Diagnostic Techniques for Bovine Mastitis

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Abstract | Bovine mastitis is the most prevalent illness in dairy cows all over the world. Furthermore, the dairy sector is one of the most important industries in many countries of the world. Globalization and liberalization are adding additional positive forces to the modern economy today, which is fast-moving forward in industrial production, and the dairy business is no exemption. Milk is a necessary social meal, but as the quality and quantity of milk decline, it has an impact on dairy farmers' livelihoods and family nutrition, resulting in significant losses. Because the quantity and quality of milk are so important in the dairy sector, there have been significant financial losses. As a result, adequate maintenance and preventative measures must be maintained in order to ensure any dairy business is viable and sustainable. Various strategies for detecting mastitis have evolved, including proteomic approaches, particular immunoassays, and infrared thermography, all of which provide quick findings. This article efforts primarily on the complex methods of mastitis detection, as identification of the etiological agents are vital to preventing mastitis in dairy cows.

Keywords | Mastitis, Diagnosis, Bovine Mastitis, Immunoassays, Diagnostic Techniques, Chronic Mastitis

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INTRODUCTION

Bovine mastitis is a multifactorial inflammation of the mammary glands in dairy cows that causes milk quality, milk output, and financial losses to dairy farmers. It is caused mostly by non-contagious environmental bacterial species and is one of the most common illnesses among dairy animals in Pakistan, especially bovines (Kabir et al., 2019).

Mastitis is a mammary gland parenchyma inflammation characterized by a variety of fleshly, biological, changes in udder glandular tissue and bacterial contamination. Dairy cows suffering from mastitis face a number of serious health issues. In addition, it has a significant impact on animal welfare, which has the highest financial cost in dairy cows due to treatment expenses and unusable milk, which results in lowering the milk output (Radinovic et al., 2019). While comparing commercial consequences of the two types, subclinical and clinical mastitis, subclinical mastitis had a greater prevalence and causes larger annual economic losses than clinical mastitis (Shaheen et al., 2016). In Pakistan field surveys of major livestock diseases have indicated that mastitis is one of the most fatal diseases (Hussain et al., 2005).

Various types of mastitis have been identified, including clinical mastitis, subclinical mastitis, and chronic mastitis

(Abebe et al., 2016). An abrupt change in milk composition, fluctuations in the arrival of the udder as a result of mild to severe indications of inflammation, and reduced milk output are symptoms of clinical mastitis. Clinical mastitis is easily detectable while subclinical mastitis cannot be seen in the udder or the milk and happens when proper treatment is not given during clinical mastitis. It is also associated with decreased milk production and an increase in somatic cell count, making it one of the most important causes of serious economic impacts.

Mastitis is caused by a number of epidemiological risk factors that have a key influence on udder inflammation. Mastitis is caused by a variety of infections and is divided into two types: contagious and environmental mastitis (Cervinkova et al., 2013). During the previous few decades, there has been a shift in the prevalence of contagious versus environmental mastitis. Pathogens that are contagious include those that transmit from cow to cow during milking, and the source might be the udder, skin, or teat canal. *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma bovis*, and *Corynebacterium bovis* are all contagious pathogens that contribute to causing mastitis in animals (Radinovic et al., 2019).

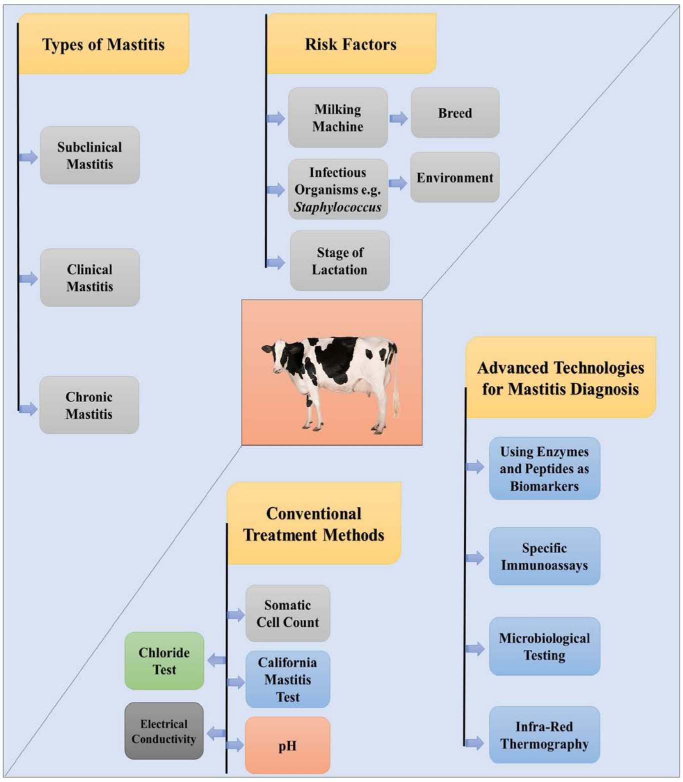
The pathogens like *Escherichia coli*, *Klebsiella* spp., *Streptococcus dysgalactiae*, and *Streptococcus uberis* are examples of environmental pathogens that cause infection during milking times. It has also been noted that infection-causing bacteria routinely induce long-term subclinical mastitis infection, which is thought to be persistent in a high number of animals, and that environmental infections are the most common cause of clinical mastitis.

Subclinical mastitis is difficult to diagnose during the early stages of infection since there are no obvious symptoms. Mastitis in milk can be detected using a variety of traditional techniques. However, somatic cell count is the gold standard for assessing mammary gland inflammation and infection severity. Simultaneously, other traditional procedures for detecting mastitis are now in use, including the California Mastitis Test, CAMP test (Christe, Atkin, Munch, and Peterson), Hotis Test, pH, and electrical conductivity. Enzyme-linked immunosorbent assays and nucleic acid-based testing have recently made considerable advancements in the identification of mastitis, although both approaches have some limitations (Viguier et al., 2009). Furthermore, biomarkers associated with the start of the illness, such as N-acetyl-glucosaminidase (NAGase), Lactate dehydrogenase (LDH), glucuronidase, and alkaline phosphatase, are measured using culturing techniques for mastitis diagnosis.

Apart from these techniques, studies are being carried out using cross-disciplinary approach in order to make the diagnosis of mastitis effective and more rapid. With the rapid increase in adaptation rates of automated milking systems by dairy farmers all over the globe, the demand for automated detection of mastitis in automated milking cows is also increasing. These automated methods should be effective enough to detect the mastitis parameters that are usually detected by the milkers during manual milking. By using cross-disciplinary approach, different disciplines including machine learning, mathematics and statistics are being utilized for mastitis diagnosis by integrating modeling. A recent study in Canada was carried out aimed to develop automated system for the detection of mastitis on the farms where automated milking was carried out for milk collection (Naqvi et al., 2022). Another emerging method for the automated detection of clinical mastitis in farms where automated milking is being carried out is the use of sensors. Many studies have shown the use of sensors as an effective mastitis detection method on farms where automated milking systems are installed (Slob et al., 2021; Fadul-Pacheco. et al., 2021). To imorove the efficacy of using sensors for mastitis detection, studies are being carried out to develop specified algorithms for detection of specific conditions of mastitis. A recent study by Hogeveen et al. (2021) followed the approach of designing specific algorithms for detection of specific clinical mastitis conditions and showed high specificity (>99.5%) and high sensitivity (>80%). Different time windows were used including 3 days before and after detection and 7 days before and after detection. However, there is still need of further studies on existing and newly developed sensor systems for evaluating their efficacy and novel test protocols should be developed in order to get the specific sensor systems certified by organistions like ICAR in Rome, Italy (Hogeveen et al., 2021). However, in Pakistan, most of the detection methods used for clinical mastitis diagnosis are quite traditional. Most of the small-scale dairy farmers are still engaged with manual milking and hence the visual method is considered to be the gold standard for the diagnosis of clinical mastitis. However, the trend of automated milking systems is gradually rising day by day and need for automated detection of mastitis is increasing in Pakistan also (Tahir et al., 2018). The integration of advanced detection methods like biosenors or recurrent neural network for cinical and subclinical mastitis by dairy farmers in Pakistan is limited due some factors including financial constraints of farmers and problems in practical implementation due to differences in epidemiology and disease presentation (Ghafar et al., 2020). The conventional or traditional methods for the mastitis detection are utilized in Pakistan which mainly include somatic cell count (SCC), California mastitis test (CMT), pH, and Surf field mastitis test (SFMT). However, due to increasing modernisation in the dairy industry of Pakistan, the mastitis detection methods are changing and now shifting towards immunobased essays, PCR, loop assisted isothermal amplification (LAMP) and the use of biosenors in the near future (Ashraf and Imran, 2018).

Humans require milk as a part of their fundamental diet because it provides all the nutritional components needed for energy and nourishment (Ajmal et al., 2015). According to estimates, the demand for milk has increased dramatically as the world's population has grown (FAO et al., 2018). Aside from other issues, mastitis is causing significant economic losses to the dairy industry in developing countries as well as developed ones. Mastitis causes decreased milk production and poor-quality milk in dairy cows due to an increase in somatic cell count (Hogeveen et al., 2001). Mastitis not only affects the udder but also has a significant impact on animal reproductive efficiency, particularly during early lactation (Naas et al., 2013).

Poor quality and quantity of milk, which is primarily impacted by mastitis, has been linked to one of the most significant causes of economic losses in the dairy business globally. Mastitis has been blamed for almost 70% of all economic losses associated with milk production. Increased inputs in the livestock sector as a result of mastitis have a negative impact on farmers' livelihood (Sharma et al., 2018). In comparison to modern and structured farms, the disorganized farms had the greatest prevalence of subclinical mastitis in dairy cows (Rajkhowa et al., 2018). As a result, diagnosing mastitis at an early stage of infection is critical in avoiding financial losses and maintaining the health of dairy cows.



MASTITIS AND ITS TYPES

Mastitis is a word used to describe an inflammation of the breast gland. Mastitis gets its name from the Greek word "Matos," which means "breast or udder," and the suffix "itis," which means "inflammation" (Ibrahim et al., 2017). It is also known as a mammary tissue inflammation in dairy cattle that is caused by aggressive germs all over the world (Ali et al., 2018). Mastitis causes physical and chemical changes in milk as a result of the leaking of blood components, serum proteins, enzymes, and salts into milk, causing a change in milk composition (Ashraf et al., 2018). Mastitis is classified as subclinical, clinical, or chronic depending on the degree of the illness.

Subclinical Mastitis

Subclinical mastitis is defined as mammary gland inflammation that does not manifest itself in milk or udder alterations (Langer et al., 2014). Milk seems normal in the event of subclinical intramammary infection (IMI), but there is a reduction in milk supply and poor milk quality (Salvador et al., 2014). The entry of leukocytes and erythrocytes into milk is the first pathogenic alteration seen in subclinical mastitis, leading to increased permeability of mammary capillaries and hence leading to an inflammatory response (Kumari et al., 2018).

Subclinical mastitis reduces milk output by 10% to 20%, resulting in milk contents with low nutritional value and making it unsuitable for processing. Thus, there is a need of developing a quick detection test for mastitis, particularly subclinical mastitis in milk at the earliest stage of infection (Salvador et al., 2014).

According to previous studies, subclinical mastitis was shown to be more common in Pakistan, and *Staphylococcus* species were found to be more abundant in mastitis than other infections (Kabir et al., 2019). So, it requires field veterinarians to use scientific management approaches and prompt treatment interventions to reduce the rates of subclinical mastitis and cope with the dairy animal production issues.

Clinical Mastitis

Mammary gland infection, swelling of the udder, redness of the udder, and changes in milk production in afflicted quarters in animals of a herd are all indications of clinical mastitis. Clinical mastitis is characterized by the development of clots and flakes with a watery consistency due to the presence of a significant number of leucocytes (Reddy et al., 2014). Mastitis can be classified as acute, sub-acute, or chronic depending on the degree of the inflammatory reaction. Clinical mastitis can develop in well-managed herds, with greater rates of occurrence in higher milk-producing animals, as determined by somatic cell counts (Hogan et al., 1990). The most prevalent infections linked with clinical mastitis are *Staphylococcus aureus*, *E. coli*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*.

Chronic Mastitis

Chronic mastitis develops when an animal receives insufficient therapy during the scientific or treatment step of mastitis. During persistent mastitis, milk has a clumpy shape, and udder edema and extreme tenderness develop. In chronic type mastitis, quarters grow hard, and antibiotic therapy typically fails. By milking machine or hand stripping, the microbes remained attached and are discharged along with milk and even drain to healthy quarters. As a result, even healthy cows are at risk from this kind of mastitis.

DIAGNOSTIC METHODS FOR BOVINE MASTITIS

Mastitis causes differences in milk structure and appearance, which affects milk value. Milk traditions from medical rooms with a high-level bodily cell count and persistent diseases give a plethora of evidence on udder condition, but early diagnosis of illness, such as subclinical mastitis, remains a serious difficulty. These traditional experiments are established on variations in milk structure, such as pH, chloride content, electrical conductivity, catalase substance, causal microorganism detection, bodily cell count, Hotis test, and California mastitis test. In addition, the newly designed test comprises immunoassays, a proteomic approach, and infrared thermography. In smaller dairy operations, these criteria are used to detect mastitis. Because of the sensitivity and specificity limits of these approaches, there is a need to develop new and advanced diagnostic tools for mastitis. In this regard, biomarkers specific to mastitis have also been identified, which can be utilized to diagnose the disease at an early stage (Viguier et al., 2009).

Somatic Cell Count

Somatic cell count (SCC) is used as a gold standard for diagnosing subclinical mastitis and is a vital metric for the dairy sector since it influences the price of milk provided to producers. The bodily cell total is a measurement of white blood cells in milk that is used to determine mastitis resistance and susceptibility in dairy cows. SCC is used to track the degree of subclinical mastitis/ intra-mammary infection (IMI) in herds or specific cows. SCC estimate is a crucial element of milk value, cleanliness, and mastitis management (Sivaraman et al., 2021). SCC was decreased by proper milking methods, improved sanitation, the actual routine of teat dipping and dry period treatment, as well as improvements in managing practices (Savi et al., 2017).

California Mastitis Test

Schalm and Noorlander's California mastitis test has been frequently utilized for qualitative and indirect measurements of bodily cell totals (Knuth et al., 2019). This test is quick and inexpensive, and it may be used to screen for mastitis at a dairy to assess udder health and milk quality (NMC 2015). The test uses a detergent combination that interacts with somatic cells and then triggers nucleic acid to create a gel, with the strength of rainfall and viscosity indicating the number of somatic cells in milk (Barnum et al., 1961). Many studies have shown that CMT is one of the best options for determining the health state of udders because of its speed and precision (Kathiriya et al., 2009).

CMT was conducted by putting 3 mL of thoroughly combined milk test into one part of a divided plastic blade, then adding 3 mL of CMT reagent and rotating swiftly by hand for 10 times, according to Barnum and Newbould (Barnum et al., 1961). The paddle has four chambers, allowing four samples to be evaluated at the same time. The use of CMT revealed large differences in the prevalence of subclinical mastitis. CMT found a 60% frequency of subclinical mastitis in animals (Thakur et al., 2014).

According to the modified CMT score during a study, 35 percent of the animals in 60 dairy cattle from 8 different villages were infected with subclinical mastitis, and 18.25 percent of the animals were infected with quarters, which was lower than prior research (Swami et al., 2017). According to CMT scores, subclinical mastitis was found in 70.19 percent of animals and 34.94 percent of quarters (Hiitiö et al., 2017). This research-backed with Khan and Muhammad's findings, which found a prevalence of 36 percent subclinical mastitis in quarters (Khan et al., 2005). The efficacy or testing accuracy of somatic cell count (SCC) and California mastitis test (CMT) was studied in a research by Rust et al. (2021). The senstivity of CMT was found to be 76% while using the domestic detergent from the UK as a reagent. However, the specifity was found to be almost 96% in case of UK detergent as a reagent used for the CMT (Rust et al., 2021).

pH

The pH of average milk is between 6.5 and 6.7, however, when a cow has mastitis, the pH rises owing to the alkalinity of the milk. The permeability of blood capillaries improves when the mammary gland becomes swollen, allowing alkaline blood components such as sodium and bicarbonate ions to enter into milk, raising milk pH to 7.0 in severe clinical mastitis. Beneficial areas with a somatic cell count of < 1,00,000 cells/mL at 37°C had lower pH values of 6.40 and 6.60, respectively, as well as lower pH values in initial lactation (Kandeel et al., 2018). The altitude in milk pH caused by quarter swelling had a positive link with the power of the infection, where blood and extracellular fluid components combined with produced milk (Badawy et al., 2015). In clinical mastitis, a reduction in milk production rate was found due to a broken blood-milk barrier, but in subclinical mastitis, extracellular fluid components and blood were able to flow into the lumen due to leaky tight junctions of mammary epithelia (Nguyen et al., 1998). As a result, measuring milk pH might be a valuable diagnostic for identifying quarters with subclinical mastitis (SCM) or intramammary infection (IMI). The test technique was laboratory-based at the time, and this need limited the use of milk pH as a screening test for SCM and IMI (Constable et al., 2009) reported that a discrepancy in strong cation (sodium) and anion (chloride and casein) concentrations in milk caused an elevation in pH, which was then influenced by subclinical infection. Changes in milk components during the first few days after calving, as well as quarters with SCM and clinical mastitis, supported this theory (El Badawy et al., 2015). The accuracy and efficacy of pH for the diagnosis of different types of mastitis can be checked by comparing the results of pH testing with other parameters like somatic cell count, electrical conductivity, and CMT scores of the same infected cows. A study by Mahapatra et al. (2018) was carried out for testing the efficacy of these parameters alogwoth the relative oxidative stress in cows affected with mastitis. The study concluded that the results of other parameters like SCC and CMT scores should also be taken in consideration alongwith pH values for accurate detection of masttits in cows (Mahapatra et al., 2018).

Electrical Conductivity

Electrical conductivity (EC), which is based on anions and cations in milk, has been employed as a mastitis indication for the past four decades (Hamann et al., 1998). The EC is measured in Simens/cm and expressed using a portable electrical conductivity meter. The conductivity meter is cleaned with cotton before the test, and 3 mL of milk is utilized. Higher quantities of sodium and chloride ions, as well as lower levels of potassium and lactose in milk, cause an increase in electrical conductivity in infected quarters (Kitchen et al., 1981). The lactation stage, age of cow, milking interval, milk temperature, pH, and fat content are all factors that impact milk's electrical conductivity (Biggadike et al., 2002). A positive association was found between somatic cell counts and electrical conductivity, suggesting that varied factors have an impact on milk electrical conductivity (Sheldrake et al., 1983). Handheld EC meters, such as the Draminski mastitis detector, cannot be utilized to diagnose subclinical mastitis, according to (Galfi et al., 2015). Hillerton and Walton discovered that the result of electrical conductivity should be associated with the number of somatic cells in foremilk (Hillerton et al., 1991). There was a good link between somatic cell count and electrical conductivity (0.51–0.75) and inter-quarter ratios can help with evaluation (Hamann et al., 2002). Mansfeld et al., 2001) investigated the effects of several infections that cause mastitis. Quarters infected with mastitis had significantly higher EC values than uninfected quarters. Quarters infected with *S. aureus* and *Streptococcus agalactiae* had lower electrical conductivity than quarters infected with ambient streptococci, which had somewhat higher values. When these aspects are considered, electrical conductivity is effective for assessing the health of an animal's udder.

Chloride Test

Because the blood-milk barrier permeability is enhanced in mastitis-affected animals, the chloride content of their milk rises. Mastitis milk has a chloride concentration of 0.08-0.14% and mastitis milk have a chloride content of >0.14% due to inflammatory exudates (Sanders et al., 1938).

ADVANCES IN TECHNOLOGY FOR MASTITIS DIAGNOSIS

Mastitis is a complicated disorder in dairy animals across the world, with a significant influence on community strength and the financial system of any nation. As a result, there is a pressing want to establish a quick and precise solution. The most recent improvements in mastitis diagnosis are shown here.

Detection Of Mastitis Using Enzymes And Peptides As Biomarkers (Proteomic Approach)

A report on indigenous enzymes, focusing on lactoperoxidase (LPO), was first published in 1881. Since then, research has focused on the indigenous enzymes of milk. The seven different enzymes of milk discovered in the early twentieth century were LPO (lactoperoxidase), xanthine oxidase, catalase, proteinase, lipase (arylesterase), and amylase. Enzymes are catalytically active proteins that are classified as biological agents. Enzymes linked to inflammation rise, resulting in a reduction in milk production. As a result, they can be used as a biomarker to identify mastitis. A biomarker is a trait that may be tested and assessed as a predictor of normal biological processes, pathological processes, or pharmacological reactions to therapeutic treatments (Boehmer et al., 2011). Furthermore, progress has been achieved in the identification of nucleic acid indicators as well as other new biomarkers. Mastitis detection tests have traditionally relied on somatic cell count. Enzyme-based biomarkers linked to illness start might aid in the advancement of a mastitis screening test. Lactate dehydrogenase (LDH) activity in milk was shown to be a good candidate for detecting subclinical mastitis in buffaloes since it was simple to measure and had high sensitivity and specificity (Singh et al., 2016). In cows with clinical mastitis, they discovered a substantial link between lactate dehydrogenase concentrations and somatic cells. Inflammation in the udder increased somatic cell count, LDH and Alkaline phosphatase (AP) enzyme activity, and phosphorus, whereas milk calcium content decreased (Persson et al., 2017)

The considerable drop in tryptophan, kynurenine, and kynurenic acid concentrations in blood and milk in subclinical mastitis milk suggested that these components may be used as Persson biomarkers. LDH activity in milk was also measured using fluorometric and colorimetric techniques for the early detection of mastitis infection (Duarte et al., 2017). Protein biomarkers are found in milk whey during the early stages of subclinical mastitis and can be utilized as a reliable diagnostic for mastitis identification using relative proteomics (Bian et al., 2014). Difference protein term during udder infection has been studied. Proteomics was utilized to identify milk proteins that are more active during the preclinical stage of mastitis induced by *S. aureus*. Different whey protein patterns of healthy and subclinical mastitis milk were also discovered in other proteomics investigations. As a result, the proteomic analysis may be the most promising method for detecting mastitis in milk (Ryskaliyeva et al., 2018). Acute-phase proteins like serum amyloid (SAA) have been used as biomarkers in proteomic studies to identify udder inflammation (Hussein et al., 2018). The severity of mastitis and the kind of causative organisms have an influence on the biomarker selection for mastitis diagnosis because they affect the number of acute-phase proteins (APP). In comparison to other pathogens, *E. coli, S. uberis,* and *S. dysgalactiae* were reported to have higher amounts of APP in mastitis milk. CRP and Hp levels also differed considerably between clinical and subclinical mastitis without influencing MSAA3 levels (Thomas et al., 2018).

Specific Immunoassays

Another recent method for diagnosing clinical and subclinical mastitis is an immunoassay. The Hp protein was used to produce an enzyme-linked immunosorbent test (ELISA) for the diagnosis of mastitis. With SAA, the developed approach identified 0.071g/ mL of target protein (Jaeger et al., 2017).

Other indicators examined by ELISA for detection of subclinical mastitis were milk amyloid A and Sip protein (Duarte et al., 2015). ELISA can be used to diagnose mastitis by identifying new biomarkers, but it has several drawbacks, such as a limited number of antibodies available with good specificity (Hussein et al., 2018). Immunoassays, on the other hand, have some limits in terms of non-specificity owing to cross-reactions, and they necessitate specialized personnel as well as a significant investment in infrastructure (Rossi et al., 2018).

Microbiological Testing

For the identification of specific mastitis causal agents, phenotypic and genotypic methodologies were applied. In terms of cost and convenience of use, each has advantages and disadvantages. Phenotypic identification is not very precise and precise. Genotypical kits are more precise and precise. Specific culture, PCR, loop-mediated isothermal development, lateral flow tests, nucleotide sequencing, matrix aided laser desorption ionization, and mass spectrometry, among other molecular diagnostic approaches, have all been used to diagnose cancer (Chakraborty et al., 2019). The accuracy of microbiological testing in detecting mastitis is affected due to alteration in microbial populations and their detection due to the use of antimicrobials. So, there is need to evaluate the efficacy of microbiological testing for the mastitis diagnosis (Rossi et al., 2018).

Infra-Red Thermography

Infrared thermography (IRT) is nearly identical to CMT in that both tests can distinguish between clinical and subclinical mastitis. Infrared thermography is a new approach that is effective, portable, and may be used on-site to diagnose mastitis early. It is based on the difference in temperature between infected and healthy udders. Thermal cameras produce heat pictures, which are then analyzed to determine the degree of udder infection (Sathiyabarathi et al., 2016). Infrared thermography was tested to see if it had a good association with somatic cell count. Similarly, because IRT is a mobile-based application, Sinha et al. (Sinha et al., 2018) found it to be very sensitive and farmer-friendly. It can detect even tiny variations in the temperature of the udder surface, making it useful for early-stage mastitis identification.

CONCLUSION

The incidence of mastitis has increased dramatically as milk output has increased. Mastitis has resulted in financial losses for both producers and consumers as a result of decreased milk output and quality, as well as increased veterinarian and labor costs, and is regarded as one of the most expensive illnesses. Subclinical mastitis, among the several kinds of mastitis, has significant economic ramifications since it is difficult to diagnose at an early stage of infection. As a result, many mastitis diagnostic tests are employed to assess the quality of milk, but as new techniques emerge, there is a need for training and methodological understanding to interpret the results. New technological breakthroughs in mastitis detection, such as a proteomic method, can aid in the development of a possible biomarker that can be used to treat herd animals at an early stage of illness. Other approaches, including infrared thermography and ELISA, yielded reliable findings in both preclinical and clinical mastitis, according to the research cited above. These latest advancements, however, have limits and necessitate the use of a competent individual, although these unique ways have shown potential and offer advantages over old methods.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

NOVELTY STATEMENT

According to our current knowledge, no other review has been conducted in detail discussing the advanced and conventional mastitis detection techniques.

AUTHOR'S CONTRIBUTION

Analyzed the data: AK, MS & MHR, ALB, KRB. Contributed in data analysis: GSB MU, MK, Wrote the paper: AK & MHR.

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