

Bovine Mastitis: A Review

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Abstract: Bovine mastitis refers to the inflammation of mammary gland causing physical, chemical and microbial changes, characterized mainly by an increase in somatic cells in the milk and by pathological changes in the mammary tissue. The main causative agents in cattle responsible for mastitis are staphylococci and streptococci. The environmental pathogens *Escherichia coli* and *Streptococcus uberis* are becoming important causes of clinical mastitis originating from the cow's environment. Mastitis can be sub clinical or clinical in nature, leading to high economic losses in the dairy industry. This inflammatory disease has been known to cause a significant reduction in productivity, affecting both the quality and quantity of milk and also causing culling of animals at an unacceptable age. Mastitis control programmes focused on different aspects of dairy farming such as feeding practices, animal husbandry, hygiene and general health care are expected to reduce the incidence of udder infections. In addition, treating infection with antimicrobials can assist to eliminate or at least decrease the incidence of mastitis infection.

Key words: Diagnosis • Economic loss • Mastitis • Milk quality • Pathogens

INTRODUCTION

The term "Mastitis", commonly used for the inflammatory reaction in the mammary gland, is derived from the Greek word elements '*masto*' referring to the mammary gland and '*itis*' referring to inflammation [1]. This inflammatory reaction is due to microbial infection in the teat canal and mammary tissue, although mastitis could technically be used to describe any udder injury resulting in inflammation. The bacteria invading teat canal and mammary glands multiply and produce toxins that cause injury to the milk secreting tissue and physical

trauma, which reduces the yield and adversely affecting the quality of milk and milk products. The teat end plays a major role in the first line of defense against invading micro-organisms. A sphincter of smooth muscles surrounds the teat canal from outside and keeps the canal closed when not in use [2]. It restricts the entry of bacteria into the teat and also prevents milk from escaping. The inside of teat canal is lined with keratin and damage to keratin has been linked to increased susceptibility of teat canal to bacterial invasion [3]. The fibrous proteins of keratin have been reported to bind electrostatically to mastitis causing pathogens, thus

altering the bacterial cell wall and rendering it more susceptible to osmotic pressure. This disturbance in osmotic pressure causes lysis of invading pathogens [3]. Thus, the keratin structure plays a significant role in trapping the invading bacteria and prevents their migration into the gland cistern. A pre-pubertal healthy mammary gland comprises of sterile environment prior to colostrogenesis. However, at the time of milking the bacteria present near the opening of teat may enter the teat canal, causing trauma and damage to the keratin or mucous membranes lining the teat sinus [4, 5]. The pathogens are believed to freely enter into the teat canal post milking when the teat canal may remain dilated for one to two hours [6]. Following invasion microorganisms penetrate the teat canal and multiply in milk-producing tissues leading to infections.

Owing to population growth, the global milk consumption has been growing in average 10-15 million tonnes per year. The largest milk consumers, European Union and North America and several Asian countries especially China and India have a high demand for dairy products. An increase of 25% on world milk demand is expected between 2007 and 2020 [7]. Dairy cattle in a commercial milking environment need to be in perfect physical condition so as to provide high milk yield. However, the risk of lesions and infections in modern dairy farming has been alarming. Mastitis accounts for the largest economic losses on dairy farms in many countries in the world, including the USA, United Kingdom, Europe, Australia and South Africa [8], accounting for long-term reduction in milk yield [9]. Permanent loss of production in individual cows, discarded milk following antibiotic therapy, early culling of cows, veterinary costs, drug costs, increased labour, death of per acute cases and replacement costs, all contribute to heavy financial loss [10, 11]. Bishi reported the economic loss from clinical and subclinical mastitis in Addis Ababa milk shed to be approximately 270 Ethiopian birr (ETB) per lactation [12]. Mungube estimated the economic loss from mastitis in the periurban areas of Addis Ababa as 210.8 ETB/cow/lactation from which milk production loss contributed to 38.4% [13]. Mungube *et al.* in another study also reported an average loss of 17.2% in milk production due to mastitis [14]. It is estimated that in the United States the cost related to mastitis on dairy farms is approximately US\$ 200 per cow/year, which causes an annual loss of 2 billion dollars [15]. In India, higher loss of Rs. 1695 per cow was reported in indigenous cows as compared to a loss of Rs. 1597.64 per cow in cross bred cows as a result of mastitis [16]. Kappeli *et al.* also

highlighted considerable economic loss to dairy industry as a result of staphylococcal mastitis in cattle [17]. Mastitis has been ranked as number one disease of dairy animals in field surveys of major livestock diseases [18]. Thus, efforts are continuously being pulled to improve udder health and decrease the incidence of udder infection and inflammation in dairy herds. This review highlights the importance of mastitis and the related diagnostic aspects to combat the disease.

Types of Mastitis: The combined interaction of host, microbial agent and environmental factors is responsible for the development of mastitis. Depending on the extent of inflammation, mastitis can be classified into sub-clinical, clinical and chronic forms, which in turn depends on the nature of the causative pathogen and age, breed, immunological health and lactation state of the infected animal [19].

Subclinical Mastitis: Subclinical mastitis is mostly asymptomatic and represents infection without apparent signs of local inflammation, although transient episodes of abnormal milk may appear. This form of mastitis is accompanied by decrease in milk production and increase in SCC, having greater impact in older lactating animals than in first lactation cows. Subclinical mastitis is most commonly associated with *Staphylococcus aureus* and *Streptococcus species* [20]. Diagnosis of subclinical mastitis is based on testing milk for somatic cell counts. Normal udders show no outward signs of a pathological condition and the milk is free from pathogenic organisms with a normal cell count [21]. Somatic cell count as low as 1, 00 000 cells/ml has been linked to decrease in milk quality with no inflammatory changes. SCC has been found to be positively correlated with the presence of infection and a negative relationship generally exists between SCC and the milk yield [18]. Thus, the loss of milk from inflammation is inversely proportional to the individual cow SCC; as SCC rises, milk production decreases. This loss in yield can be highly significant if an IMI occurs in early lactation and persists as a chronic infection throughout lactation. There is a plethora of evidence that the dairy cow milk has a natural level of 100, 000-150, 000 somatic cells/ml and higher SCC indicates secretory disturbance [22].

Clinical Mastitis: Due to the absence of any visible indications sub clinical mastitis is difficult to detect. However, abnormalities of the udder and secretion are readily observable in clinical mastitis. There may be

sudden onset of swelling and redness of the udder accompanied by pain and altered milk secretion from the affected quarters. The most obvious abnormalities include changes in the milk, such as flakes, clots and a watery appearance. Systemic symptoms including fever, loss of appetite, reduced rumen function, weakness and depression may also be present. Chronic mastitis lasts longer resulting in persistent inflammation of the mammary gland and may be classified as peracute, acute, sub acute or chronic. Clinical cases showing only local signs are referred to as mild or moderate. The case is considered as severe if the inflammatory response includes systemic involvement such as fever, anorexia, shock. Rapid onset is however termed acute or severe mastitis. Severely affected cows tend to have serous secretions in the affected quarter. Typically only one quarter will display clinical mastitis, although any number of quarters can be infected simultaneously in subclinical mastitis. *Mycoplasma*, however, is reported to affect multiple quarters with clinical episodes. Acute mastitis shows severe clinical signs of fever, depression and loss of appetite. The most commonly associated organisms include coliforms such as *Escherichia coli* and *Klebsiella* and *Streptococcus species* [23]. The udder is swollen, hard and painful and the milk may be watery with clots or flakes. Gangrenous mastitis can also occur, particularly when subclinical, chronic infections of *Staphylococcus aureus* become severe at times of immune dysfunction such as parturition. The symptoms include anorexia, dehydration, depression, fever and signs of toxemia, sometimes leading to death. Early in the disease, the gland is red, swollen and warm and within a few hours the teat becomes cold. The secretions become watery and bloody and mammary gland finally becomes necrotic. Chronic mastitis, most commonly associated with coagulase-negative staphylococci, *S. aureus*, *S. uberis*, lasts longer and leads to persistent inflammation [24]. Clinical signs of an acute infection reappear from time to time with no clinical signs for prolonged intervals. If the infection persists for at least two months, the infection is termed chronic. Many of these infections, once established, persist for entire lactations or the life of the animal.

Prevalence: The most common infectious agents of mastitis can be broadly grouped as contagious pathogens, environmental bacteria, opportunistic bacteria and other organisms that cause mastitis less frequently [25]. Contagious microorganisms are the primary source of infection between uninfected and infected udder

quarters. They are usually found on the teat surface of infected cows and get transmitted during milking. The most frequent contagious organisms include *Staphylococcus aureus* (coagulase positive staphylococci) and *S. agalactiae* while the less common ones are *Corynebacterium bovis* and *Mycoplasma bovis* [26, 20]. In contrast, environmental pathogens are those which are present in the immediate surroundings of the cow. The primary source of environmental pathogens is the bedding used to house cattle. Contaminated teat dips, intramammary infusions, water used for udder preparation before milking, water ponds or mud holes, skin lesions, teat trauma, milk fed to calves and flies are also potential sources of infection. These bacteria include streptococcal strains other than *Streptococcus agalactiae*, such as *Streptococcus dysgalactiae*, *S. uberis*, *S. bavis*, *Enterococcus faecium* and *E. faecalis* and coliforms such as *Escherichia coli* and *Klebsiella pneumoniae* [27]. Olde Riekerink *et al.* reported *Streptococcus uberis* as the most frequent bovine udder pathogen among *Streptococcus* spp [28]. According to Bradley, *Escherichia coli* and *Streptococcus uberis* are now the two most common causes of bovine mastitis [29]. Jánosi and Baltay studied the environmental mastitis by *Streptococcus sp* and *E. coli* with a prevalence of 12.8 and 6.8 %, respectively [30]. Opportunistic pathogens include coagulase-negative staphylococci and are responsible for mild forms of mastitis. This form of mastitis becomes established if the immune system of the host is compromised or if sanitation and hygiene is not adequately practiced [31]. In general, coagulase-negative strains are regarded as non-pathogenic [26]. These staphylococci are commensals, may be isolated from milk and illicit a minor immune response in cattle causing slight infections. These include *Staphylococcus epidermidis*, *S. saprophyticus*, *S. simulans* and *S. chromogenes* [32]. Some less common bacteria and even yeasts may be responsible for causing mastitis followed by increased exposure. When wet, rainy conditions prevail in the summer months in European countries, a condition known as “summer mastitis” occurs commonly. It is more common in non-lactating cows and the source of infection is usually traced to increased exposure of cows to flies in pastures that transmit infecting *Arcanobacterium pyogenes* and *Peptostreptococcus indolicus* strains [33]. Mastitis caused by *Pseudomonas aeruginosa* has been linked to contaminated water sources and results in a condition quite similar to coliform mastitis infections where endotoxemia occurs. Quinn *et al.* also reported severe cases of mastitis due to *Nocardia asteroides*

infection from soil resulting in fibrosis and permanent damage to mammary tissues along with some rare infections due to *Bacillus cereus*, *Streptococcus pyogenes* and *S. pneumonia* that cause acute mastitis accompanied by fever in the host [26].

Coagulase-negative staphylococci (CNS) are reported to be frequently associated with bovine mastitis. Waller *et al.* studied the prevalence of CNS species in clinical and subclinical mastitis and reported inter-species variation of pathogenicity and epidemiology [34]. *Staphylococcus chromogenes* and *Staphylococcus epidermidis* were found to be the most common CNS species found followed by *Staphylococcus simulans* and *Staphylococcus haemolyticus*. In a study conducted by Girma *et al.* in Ethiopia, *Staphylococcus* species (47.11%), *Streptococcus* species (31.40%) and coliforms (9.92%) were reported as the predominant bacteria in positive samples [35]. Cows in their first three months of lactation were found at higher risk of acquiring mastitis that decreased as lactation stage increased, which is in accordance with previous reports [36-39]. According to Quinn *et al.*, the occurrence of more cases during earlier lactation stage has been attributed to the absence of dry cow therapy and birth related influences [40]. Kerro and Tareke and Radostitis also suggested higher susceptibility of mammary gland to new infection during early lactation and late dry period which was linked to absence of udder washing and teat dipping, thus increases the number of potential pathogens on the teat surface [37, 41]. High prevalence of mastitis was reported in animals kept on farms with poor hygienic conditions and poor milking hygiene [42]. Some workers reported 85.7% prevalence of mastitis associated with udder/ teat injuries as a result of unsatisfactory udder management and health [43]. In India, Joshi and Gokhale reported subclinical mastitis to be more prominent (varying from 10-50% in cows) than clinical mastitis (1-10%) and the monsoon season was more prone to subclinical mastitis than summer or winter [44]. They observed increased prevalence with higher lactation number with animals in fourth to fifth month of lactation were found to be more susceptible (59.49%). Also, hind quarters were found more affected (56.52%) than fore quarters (43.47%).

Diagnostic Aspects of Subclinical and Clinical Mastitis: Mastitis management is primarily focused on preventive measures along with prompt identification and treatment of disease. The first step in diagnosing mastitis is visual observation of the udder and milk through forestripping [45]. A number of screening tests are available for timely

diagnosis of sub clinical mastitis. However, these tests are indirect methods for detecting the presence of inflammation or identifying microbes as they primarily determine the concentration of byproduct of inflammatory response [46]. Early detection of most cases of mastitis depends on stripping milk from a cow and examining it prior to milking as changes in milk (clots, flecks, changes in colour or consistency) are often the first sign of mastitis. Udder swelling, redness, hardness, heat and pain caused by mastitis constitute the first line of mastitis detection. Unfortunately, the udder changes are detectable fairly late causing delayed treatment and increased risk of disease spread. Following are some of the diagnostic techniques frequently used to test bovine mastitis.

Electrical Conductivity: Inflammation and tissue damage caused by mastitis leads to infiltration of blood components into milk thus changing the proportions of milk from infection free udders. There is an elevation in sodium and chloride ions in mastitis infected milk thus making it a better conductor of electrical current. As a result, the electrical conductivity of milk increases due to increase in Na^+ and Cl^- and decrease in K^+ and lactose [47]. A healthy cow is reported to maintain a very consistent level of conductivity with drastic spikes if the udder becomes infected. Thus, it is essential to know the cow's normal level of conductivity so as to notice any changes subjected to the disease. The conductivity changes are believed to develop 24 to 36 hours before visible symptoms, thus detecting clinical mastitis in advance of visible changes in foremilk or udder tissue. However, due to the absence of a single threshold, different cows have different ideal or normal conductivities. Also, individual quarter tests are needed as whole cow conductivity is not sensitive enough and changes in an affected quarter can easily be swamped by the lack of change in the other three quarters. Thus, a dedicated computer programme is required to deal with all the data. At last, considering a minimum false positive rate of <0.5% per test indicates that two cows will be wrongly flagged as having a new case of mastitis every milking in a 100-cow herd. Therefore, despite of the merit of measuring milk conductivity for early mastitis detection the actual success depends on reliability of equipment, standardization of sampling procedure and appropriate analysis of the data. With wide availability of hand held conductivity testers for checking suspect cows and also the commercial systems that incorporate conductivity sensing directly into the milking cluster mastitis detection

is simplified. Software routines are available to monitor the incoming data and compare it to historical benchmarks, which is of great help in detecting significant changes and planning further evaluation.

California Mastitis Test: The California Mastitis Test (CMT), also known as rapid mastitis test, Schalm test, or the Mastitis-N-K test, is considered as the most reliable, simple, fast, easy to use and economical cow side test for the detection of subclinical mastitis. The test is based on the reaction of a detergent containing CMT reagent with DNA of cell and pH indicator (Bromo cresol purple) that changes color in response to increase in pH value of milk from the normal 6.6 to 6.8 or above [48]. This happens due to increase in the number of leukocytes and alkalinity of milk due to infection [49]. The CMT reagent lyses the cells, gels the DNA and the degree of gel formation indicates infection. According to Chauhan and Agarwal [49], the test is subjectively read as negative/trace, +1, +2 and +3 and the results read as: Negative Liquid milk with no streak or precipitation, + as Weak positive, streaky fluid, ++ as Slimy and +++ as Gelatinous. Maximum gel formation occurs within 2.5 minutes. Thus, the amount of DNA in milk secretions is qualitatively estimated with a direct correlation between DNA and white blood cells in milk. The CMT plays a significant role in detecting subclinical mastitis but serves little purpose in acute clinical mastitis. Also, recently fresh cows and cows at the end of lactation just before dry-off tend to have a high CMT score. Discrepancies may arise among evaluators as the CMT results are qualitative and loss of production correlates directly with CMT scores. 25 to 50% production loss from quarters with CMT +3 values has been reported [26]. The CMT can be extremely valuable in identifying cows with subclinically infected quarters for herds that do not perform individual somatic cell count on a regular basis. This information can be entered into a spreadsheet to track individual cows and also calculate approximations for infection risk at the herd level.

Somatic Cell Count: A strong and directly positive relationship has been stated between the somatic cell count (SCC) of the quarter samples of milk and the milk yield marking intra-mammary infection of the quarter. Radostits *et al.* highlighted that the distribution of somatic cell count in a herd is a reflection of intra-mammary infection [48]. Somatic cells constitute both leucocytes (75%) and epithelial cells (25%) and the number of cells reflects the severity of mastitis [50].

Somatic cells migrate into the udder in large numbers quite early during mastitis, making SCC of milk a useful measure of infection status. Also, a high cell count hints towards the onset of a clinical case. In response to bacterial invasion the damaged milk-secreting cells release substances that increase the permeability of vessels and attract leukocytes so as to engulf and destroy the invading bacteria. The leukocytes further recruit more of their kind from the blood into the milk and if the targeted bacteria are not entirely destroyed, these leukocytes continue to multiply and begin to invade smaller ducts and alveolar areas. Often the invading pathogens are eliminated rapidly and the infection is cleared, leading to opening of clogged ducts and normalization of milk composition and production within a few days. However, if this fails to happen and the infection persists, the ducts remain clogged and the entrapped milk makes the secretory cells revert to a resting (non-producing) state along with shrunken alveoli. This further leads to complete destruction of alveolar structures, which are then replaced by connective and scar tissues. Thus, the number of somatic cells in the milk elevate as the disease progresses, causing permanent reduction in milk yield [19]. Timely monitoring of cell count concentrations determines infection status changes and marks as one of the best-developed and most widely used practice for estimating udder health. Electro-optical Fossomatic method is considered as the standard method for somatic cell counting among various automatic methods. The threshold of 500000 cells/ml for a quarter has not been relevant for a long time and has been revised as 200000 cells/ml [51]. A healthy quarter with no bacterial growth has been reported to contain less than 100000 cells/ml and the quarter is very likely to be infected with a cell count exceeding 200000 cells/ml. The minor pathogens have been reported to cause less elevation in the SCC as compared to the major pathogens [22]. Djabri *et al.* studied the effect of different pathogens on quarter SCC and found average SCC for bacteriologically negative quarters to be 68000 cells/ml, for quarters infected with minor pathogens between 110000 to 150000 cells/ml and for quarters infected with major pathogens to be higher than 350000 cells/ml [52]. They also reported the highest mean value of over 1 million cells/ml in mastitis could be caused by coliforms and *Streptococcus uberis*. Hogeveen *et al.* observed decrease in bulk SCC with a shift from two times a day to three times a day milking [53] while very short milking intervals (4 h and less) were found to increase SCC [54-55].

Bioluminescence Determination Assay: The bioluminescent assays are alternate ways to determine somatic cell count in milk, based on determination of ATP from somatic cells ([ATP_{som}]). Frundzhyan *et al.* developed a revised version of bioluminescent SCC assay to quantify total non-bacterial ATP concentration in milk [56]. They observed that the novel ATP-releasing agent Neonol-10 (oxy-ethylated iso-nonyl phenol) at a concentration of 1.5% w/w led to 100% lysis of somatic cells while not disrupting the bacterial cells in milk. This method is highly efficient (capable of detecting as low as 900 cells/ ml), takes 2-3 minutes to analyze a sample and also suitable for on-site mastitis diagnosis.

NAGase Test: Many enzymes have been suggested to increase subjected to mastitis with possible potential in mastitis detection. Ball and Greer investigated the use of N-acetyl-beta-D-glucosaminidase (NAGase) test for detecting subclinical mastitis in surveys of milk samples from 20 farms and reported an average of 16.6 per cent false positives and 2 per cent false negatives per herd [57]. They found direct correlation between NAGase concentration and the presence of pathogens and clinical infections. Berning and Shook examined NAGase activity in milk to changes in bacteriological status in 550 cows in 10 commercial herds and found that natural logarithm NAGase and log cell count were most responsive to changes in bacterial status [58]. The log NAGase was reported to be more effective in identifying major from minor pathogen infections while log SCC was better suited to differentiate between infected and uninfected classes. Likewise, Kitchen *et al.* reported changes in the level of the tissue damage marker enzyme NAGase in quarter foremilk in accordance with the presence and type of pathogenic bacteria and somatic cell counts [59]. Minor pathogens (coagulase-negative staphylococci, *Corynebacterium bovis*) were found to elicit a mild SCC increase in infected quarters with marginal tissue damage and mean NAGase activity increasing from 21 in healthy quarters to 28 in infected quarters. Also, major pathogens (i.e. *Staphylococcus aureus*, *Streptococcus agalactiae*, *S. dysgalactiae* and *S. uberis*) were found to cause more severe tissue damage with mean NAGase of 48 and rise in SCC. They concluded that combining SCC and NAGase data possibly gives a more definite diagnosis of bovine mastitis. Hovinen *et al.* investigated the NAGase activity of 808 milk samples from healthy quarters and quarters of cows with spontaneous subclinical and clinical mastitis and studied the associations between milk NAGase activity and SCC [60]. The results indicated a high level of

accuracy (0.85 and 0.99) in detecting both subclinical and clinical mastitis. It was also recommended to determine NAGase activity from quarter foremilk after at least six hours from the last milking, samples be frozen before analysis. A fluorometric NAGase assay based on microtitration tray technology has been developed [61]. Although this technique has potential for herd monitoring, no test is commercially available. Preliminary results from electrochemical method for determination of milk NAGase activity have been published and described above but further research is needed for quick and reliable detection of mastitis.

Trypsin Inhibition Activity: Inflammation within the mammary gland can be very well detected by assessing the integrity of the mammary epithelium. The inflammatory reaction in mastitis is associated with increased permeability between blood and milk compartment leading to leakage of blood plasma proteins into the milk. Buzalski and Sandholm suggested milk α 1-antitrypsin (α 1-protease-inhibitor) as potential indicator of mastitis as low molecular weight proteins in blood plasma easily cross the blood-milk barrier [62]. Thus, the presence of antitrypsin reflects leakage of blood protein blood plasma into milk. The biggest advantage of this method is that its high capacity and sensitivity permits whole herds to be analyzed on a quarter basis to identify inflamed quarters during a single sampling. However, traces of colostral antitrypsin can be seen in colostral and postcolostral samples upto 1 month after parturition [63] which constitutes the major limitation of the present assay. Sandholm *et al.* have reported a close agreement with BSA and somatic cell counts [63]. Also, increase in lactation number was found to slightly affect antitrypsin and BSA concentrations while somatic cell content was markedly affected [64]. Colorimetric procedures have been developed to measure milk antitrypsin and hooked to computerized readers such as the Multiskan MC reader to interpolate antitrypsin from the standard curve and analyze results. These measures are often compared with bacteriologic study of milk to set criteria for automatic identification of mastitis in individual quarters. Thus, the colorimetric procedure is considered an efficient system for large scale monitoring of milk antitrypsin activity, using microtitration plates and the Multiskan system. Thus, the α 1-trypsin inhibitor is quantified indirectly by measuring the trypsin inhibitory capacity of the milk where the remaining trypsin activity is measured using colorimetric or fluorometric substrates.

Acute Phase Proteins: Acute inflammation generates an acute phase reaction that causes release of many proteins synthesized by the liver, known as positive acute phase proteins or simply acute phase proteins [65, 66]. Bovine serum albumin was the first acute phase protein measured from milk as an indicator of inflammation [63]. Haptoglobin and serum amyloid A (SAA) are considered as the most sensitive acute phase proteins in cattle depicting a substantial rise (over 100-fold) in response to acute inflammation while α_1 -acid glycoprotein has been reported to relatively increase in chronic conditions [67]. Ohtsuka *et al.* reported that the concentration of haptoglobin in serum increased dramatically in cows with experimental and spontaneous coliform mastitis [68]. Analytical methods have been developed to quantify serum haptoglobin with a biochemical assay and SAA with an immunoassay [69]. Research has been marked towards determining acute phase proteins in the milk for early detection of mastitis. The biggest advantage of these proteins is complete absence in healthy cows. Eckersall *et al.* measured haptoglobin and SAA from milk and serum and tried to draw comparison to detect IMI [69]. They found a significant correlation between haptoglobin concentration in serum and milk while the concentration of SAA was not related. However, no correlation was drawn between haptoglobin and serum amyloid A levels in milk. Serum amyloid A was reported to successfully distinguish between mild and moderate mastitis [65] and may be synthesized extrahepatic as well as locally in the mammary gland [69]. Eckersall *et al.* [69] developed tests with high specificity (100%) with no false positive results and a reasonable sensitivity (86 and 93) for the diagnosis of mastitis, with a threshold value of 0.02 mg/ml for milk haptoglobin and 0.55 mg/ml for milk SAA. The SAA assay is often disadvantageous as an ELISA test as the haptoglobin assay is faster and more easily adapted to automatic analysis systems.

Biosensors for Mastitis Detection: Biosensors for mastitis detection have been developed for on-site testing in an attempt to develop less lengthy approaches over conventional diagnostic methods. Recent advances in nanotechnology and biotechnology have led to the development of analytical tools called biosensors, capable of converting the biological compounds in a sample into electrical signals. These signals can detect the presence of particular cells and markers with high sensitivity upon proper tuning and amplification. The biological element known as bioreceptor interacts with a physical transducer called the sensor and produces a measurable signal that is transformed into data. The most commonly used

recognition elements in sensing bacterial contamination include single stranded oligonucleotides, antibodies and enzymes followed by peptide nucleic acids, bacteriophages and artificial binding proteins [70]. The development of smaller, cheaper and sensitive on-farm testing systems thus simplifies the complex molecular assays for diagnosis of the disease. Biosensors are reliable and require less time to process results, can be automated and untrained farmers may be trained quickly with new improved approaches for herd management. Many workers have stated efficient use of biosensors for detection of pathogens and other contaminants in products of animal origin [71-73]. However, the complexity of milk matrix interferes with this mechanism and thus detection of mastitis using milk as target sample has limited results. This may be improved by sample preparation such as filtration of proteins, fat and somatic cells. In addition, enzyme NAGase and haptoglobin also serve as common biomarkers for mastitis detection. Flow-based biosensors are a modified version of detection system. Peedel and Rinken quantified *S. aureus* in freshly spiked milk in just 17 minutes with the help of flow based optical immune biosensing [74]. Also, DNA amplification-based biosensing devices have been widely investigated [75].

Lateral Flow Assay: Detection of subclinical mastitis in its initial stage can save great economic losses. Neutrophils account for about 25% leucocytes in normal milk and their number increase dramatically with mastitis infection [76]. One of the major lysosomal enzymes present in azurophilic granules of neutrophils is Myeloperoxidase (MPO) and has been reported to play a significant role in innate immunity [77]. Thus, MPO in bovine blood neutrophils has been reported to serve as a novel biomarker for mastitis detection using flow cytometric method [78]. Lateral flow assay (LFA) is a simple, cost-effective and rapid on field assay that relies on antigen-antibody interactions that can easily be visualized with the help of a labelling agent [79]. Colloidal gold is the most commonly used labelling agent used to detect the presence of the analyte of interest in the sample [80]. Alhussien and Dang developed a semi quantitative lateral flow assay targeting MPO enzyme of milk neutrophils [81]. Colloidal gold nanoparticles (GNPs) were used as labeling agent and monoclonal anti-MPO antibodies were assessed using ELISA and dot blot. They standardized the conjugation method for GNP and anti-MPO antibodies, placed the conjugate over conjugate pad, coupled MPO with a carrier protein (OVA) and placed species-specific secondary antibodies on test and control

lines, respectively. 75 milk samples from healthy and infected cows were tested with the developed assay achieving >97% accuracy. MPO as low as 1.5 ng/ml was detected in the samples and no cross reactivity with other milk proteins was recorded. Owing to its high sensitivity, lateral flow assay is far suitable for detecting mastitis infection even in sub clinical stage.

CONCLUSION

The inflammation of mammary gland, known as mastitis, is regarded as one of the most costly and complex diseases of the dairy industry. Bovine mastitis is associated with huge economic consequences related to treatment, production losses, culling and changes in milk quality, thus leaving a substantial impact on the farm business. *Staphylococcus aureus* is the predominant organism among all the pathogens of bovine mastitis. Mastitis represents a major challenge to the worldwide dairy industry as the last forty years have seen a dramatic increase in clinical mastitis incidence. Milk somatic cell count constitutes a useful tool, along with other diagnostic techniques, to measure milk quality and health status of the mammary gland. Increase in somatic cell count is linear to reduction in milk yield which adversely affects both the farming and processing industry. Thus, timely detection of udder infections or abnormalities and preventive strategies are as important as timely treatment of bovine mastitis.

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