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A review on mastitis

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Abstract
Mastitis is one of the most prevalent costly diseases for dairy farmers and industry, resulting in a great deal of economic losses. Annual losses in the dairy industry due to mastitis was almost 2.37 thousand crore rupees in India. The level of infection that develops inside the udder of a cow is dependent upon many factors. Although mastitis is caused by various bacteria, viruses, fungi and prototheca, the most common cause are Gram-positive and gram-negative bacteria. Many different control measures of bovine mastitis include hygienic management procedures, antibiotic treatments, teat dipping sealants, and intramammary devises. The use of animals that are resistant to a determined disease reduces the need to apply drugs, with consequent decrease in the levels of contamination of products and the environment.

Keywords: Mastitis, economic losses, bacteria, and control measures

1. Introduction

Bovine mastitis is one of the most prevalent costly diseases for dairy farmers and industry, resulting in a great deal of economic losses, mostly because of reduction of milk yield, decreased milk quality, higher medication cost, loss of milking days, reduced milk price and increased labour (Seegers *et al*., 2003 ; Cremonesi *et al*., 2006) [50, 12]. Mastitis also affects the quality of dairy products, and reduces their shelf life. Most estimates view that on an average and affected quarter suffered a 30 % reduction in productivity and an affected cow estimated to lose 15 % of its production for lactation (Prasad, 2001) [41].

Mastitis is an inflammatory condition of the mammary tissue resulting in several metabolic and physiological changes, trauma, and more frequently it is caused by environmental or contagious pathogenic microorganisms (Oviedo-Boyso *et al*., 2007) [35], including Gram-positive and Gram-negative bacteria, algae and mycoplasmas (Zadoks *et al*., 2011) [58]. There are two prevalent forms of mastitis in terms of level of severity; clinical, and sub-clinical. Clinical form of mastitis shows visible symptoms whereas sub-clinical form does not show any visible symptoms. Clinical mastitis in a dairy herd is threatening to a farmer and treatment is given immediately to control it. But, sub-clinical mastitis cannot be identified without a laboratory or field test, mostly remains unnoticed by the farmer.

1.1 Economic loss due to mastitis

Dua (2001) [15] reported that, in India, economic loss due to mastitis was INR 6,053.21 crore, where majority of loss was found to be related to sub-clinical mastitis (70 to 80 per cent) which accounted around INR 4,365.32 crore. According to Varshney and Narsh (2004) [57], incidence of subclinical form of mastitis was found to be more common in India (varying from 10-50% in cows and 5-20% in buffaloes) when compared to clinical mastitis (1-10%). Annual losses in the dairy industry due to mastitis was almost 2.37 thousand crore rupees in India. Out of this, sub-clinical mastitis accounted for approximately 70% of the loss. As per the data from NDRI, the estimated economic loss due to mastitis was to the tune of INR 7165.51 crore per annum. Sinha *et al*. (2014) [53] stated that economic loss due to sub-clinical mastitis in crossbred cows was INR 592.87 per lactation and loss due to decrease in milk production was INR 700.18.

2. Factors influencing the incidence and aetiology of mastitis

The level of infection that develops inside the udder of a cow is dependent upon following factors:-

- Causative agents
- Genetic makeup of the animal
- Physiological status of the animal
- Number of somatic cells present in the infection stage
- Natural defence mechanism of the mammary gland

All of these factors play an important role in determining the severity of mastitis.

2.1 Causative agents

Satishkumar and Suryanarayana (2003) [48] stated that mastitis was caused by various infectious agents including bacteria, mycoplasma, virus, fungus and infections due to trauma or injury to the udder, unhygienic conditions of the animals and the barn. Mastitis caused by major pathogens was classified as either environmental or contagious. The contagious pathogens usually have a component to adhere to the epithelial cells of the mammary gland or to become intracellular, in order to secure themselves from the intramammary host defence mechanisms. *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus agalactiae* were included in this group of pathogens. Some *S. aureus* strains also have the ability to resist host natural defences and antimicrobials and they produce beta-lactamase which inactivates penicillin. The pathogens which caused mastitis through environment were included a vast number of Gram-positive bacteria *viz*., *S. equinus*, *S. uberis*, *Enterococcus faecium* and *Enterococcus faecalis*. *Escherichia coli*, *Klebsiella*, *Serratia*, *Enterobacter* and *Pseudomonas spp.* are of the most common Gram-negative

environmental pathogens of the bovine mammary gland.

2.2 Genetic makeup

Bramley *et al*. (1981) [7] observed that challenging of cows with live *E. coli* leads to intramammary infection and shows huge variations in the resistance to mastitis among the twenty animals. They also found that the rate of infection was significantly lower when teat contamination was applied one hour before milking compared to contamination applied immediately after milking.

Capuco *et al*. (1992) [9] examined Jersey cow challenged with bacteria, after removing keratin from the teat. They found that rate of infection was greater in keratin removed quarter than normal quarter and concluded that the physical appearances of the teat end, shape, and amount of keratin found in teat canals were associated with susceptibility to mastitis.

Detilleux (2002) [13] determined the genetic associations governing the severity of mastitis by parameters such as serum concentration of the immunoglobulins (IgG and IgA), the number of polymorphonuclear neutrophils (PMNs), and somatic cell count (SCC). The results suggested that these parameters varied from one animal to another and could influence their resistance to infections.

Joshi and Gokhale (2006) [24] suggested that the genetic makeup of animals play an important role in determining susceptibility to mastitis and it's differ from one breed to another. They found that the occurrence of mastitis depend upon different factors like herd size, variations in socio-cultural practices, climatic conditions of the region, milk marketing, system of feeding, and management. They also observed that the incidence of mastitis was maximum in Holstein and Jersey breeds and lowest in native breeds of cattle and buffaloes.

2.3 Physiological state

McDonald and Anderson (1981) [31] reported that the susceptibility of cows to mastitis could be overwhelmed by the season, parity and physiological stage. Gonzalez *et al*. (1990) [18] indicated that during the lactation cycle, drying off period of cow was the most susceptible phase for the exposure to pathogens. At drying off period, bacteria were not regularly being flushed out by milking and therefore, it was much easier for the pathogen to spread an infection inside the mammary gland.

Chew *et al*. (1985) [11] studied the susceptibility to mastitis due to nutritional deficiency. It has been reported that nutritional deficiency increased the susceptibility to mastitis, and supplementation of vitamin A, vitamin E and selenium might reduce mastitis incidence in the animal. These nutritional factors might alter the susceptibility of mastitis through their impacts on the immune responses of the animals.

Madsen *et al*. (2002) [30] stated that during transition period, the immune system was suppressed due to hormone changes at calving to meet the high energy demands during elevated milk production and reduced feed intake which favoured more intramammary infections.

2.4 Somatic Cell Count

The somatic cell count (SCC) plays an important role in protecting the mammary gland against infectious diseases (Bradley, 2002) [6]. When infection started, a large number of neutrophils were migrated from the blood stream via diapedesis into the milk and account for more than 90% of the SCC (Sordillo *et al*., 1997) [54]. The rate at which neutrophils were activated and migrated to the site of infection determined the severity of the infection. Weak recruitment of neutrophils at the site of infection was also believed to result in a higher susceptibility of mastitis (Shuster *et al*., 1996) [52]. Once the neutrophil reaches at the site of infection, it will exert their bactericidal functions, mainly through phagocytosis and the respiratory burst, to destroy the invading bacteria (Sordillo *et al*., 1997) [54]. It has been described that cows with a higher SCC are less susceptible to infections in comparison with cows with low SCC (Schukken *et al*., 1999 and Peeler *et al*., 2000) [49]. Under normal conditions, the SCC should be less than 200,000 cells/ml (Miller *et al*., 1999) [32].

Piccinini *et al*. (1999) [40] reported that dysfunction of neutrophil activity had been associated with high incidence of intra-mammary infection. They also found that decreased respiratory burst in neutrophils, increases the susceptibility of the mammary gland to invading pathogens. Dosogne *et al*. (1998) [14] also found that acyloxyacyl hydrolase activity in neutrophils increases the susceptibility of the mammary gland to invading pathogens.

During infection, the rapid influx of neutrophils, followed by macrophages and lymphocytes are recruited into the mammary glands. Paape *et al*. (2003) [38] reported that the function of the macrophage is to actively participate in the removal and replacement of the neutrophils. The numbers of macrophage and lymphocytes are much less elevated in comparison to neutrophils during the early stage of an infection.

Bannerman (2009) [4] suggested that the inflammatory response was described by an influx of somatic cells mainly of neutrophils, lymphocytes and macrophages, thereby speed the host's immune response against the invasive pathogens.

2.5 Defence mechanisms in the mammary gland

Proinflammatory cytokines are known to be important in the defense mechanism of the mammary gland that will elicit the acute-phase response and allow an accumulation of leukocytes at the site of infection (Riollet *et al*., 2000) [46].

The mammary gland also has complement system, with effector functions that include recruitment of phagocytes at the site of infection and opsonisation and killing of pathogens (Rainard, 2003) [42]. Phagocytes like macrophages and neutrophils can recognize, ingest and eliminate the invading micro-organisms (Paape *et al*., 2002) [37].

The mammary gland contains several chemical substances like lactoperoxidase, lysozyme, antimicrobial proteins like lactoferrin and transferrin, and antimicrobial peptides defensins which inhibit the microbial growth (Rainard and Riollet, 2006) [43]. Taken all together, the innate immune response makes an important first line of defence against the microbial infection but also has a crucial role in the activation of adaptive immunity.

3. Important bacteria causing mastitis

Although mastitis is caused by various bacteria, viruses, fungi (Farnsworth, 1977) and prototheca (Jensen *et al*., 1998) [23], the most common cause are Gram-positive and gram-negative bacteria. The immune response of the mammary gland varies towards different bacterial infection (Bannerman *et al*., 2004; Lee *et al*., 2006) [3, 29]. The two most common bacteria which

cause mastitis are *Escherichia coli* (Hogan and Smith, 2003) [21] and *Staphylococcus aureus* (Bannerman *et al*., 2004) [3]. Barkema *et al*. (1998) [5] also reported that *S. aureus* and *E. coli* are the most prevalent pathogens in bovine mastitis.

3.1 Mastitis caused by *E. coli*

Among all the coliforms causing bovine mastitis, *E. coli* is one of the most common in infections in comparison to other Gram-negative rods such as *Enterobacter* and *Klebsiella* in bovine mammary glands. Coliform bovine mastitis accounts for comparatively 24-40% of c1inical cases (Paape *et al*., 1996) [36]. *Escherichia coli* has the ability to persist after an infection and reappear after several months (Bradely, 2002) [6]. Ren *et al*. (2004) [44] demonstrated that *E. coli* has the ability to synthesize biofilms, a polysaccharide matrix meshwork that is required for the transfer of nutrients and for the removal of wastes, in order to persist undetected thus allowing the bacteria to survive. Biofilms are synthesized early-on in the infection. *E. coli* express a variety of virulence factors, but no coherence between the severity of disease and specific virulence factors could be defined (Suojala *et al*., 2011) [56].
During the bacterial multiplication, destruction and lysis of *E. coli*, a potent endotoxin called lipopolysaccharide (LPS) was released which stimulate the activation of defense mechanisms as well as being a toxic compound to the mammary epithelial cells. It has been well reported that a cell wall component of Gram-negative bacteria, LPS is the key virulence factor eliciting the clinical symptoms associated with *E. coli* infections (Bannerman *et al*., 2003) [2].

3.2 Mastitis caused by *S. aureus*

Staphylococcus aureus, a Gram-positive coccus, has been the most predominant contagious pathogen of bovine mastitis with a characteristic pathogenicity and poses serious problems to the dairy industry as well as drawing public concerns. According to Natzke *et al*. (1972) [33], around 19 to 40% of cows were infected with this organism and that infected cows produce less milk as compared to non-infected cows.
Mastitis caused by *S. aureus* predominantly cause subclinical mastitis resulting in chronic infection that can pursue for the life of the animal (Riollet *et al*., 2000) [46]. The bacteria remain in mammary glands, teat canals, and teat lesions of infected cows and are contagious. Initially, the bacteria destruct the tissues lining the teats and gland cisterns within the quarter, which eventually leads to formation of scar tissue then the bacteria move up into the duct system and establish deep-seated pockets of infection in the milk secreting cells (alveoli). This is continued by the formation of abscesses that wall-off the bacteria to prevent spread but allow the bacteria to avoid detection by the immune system.
In Gram-negative bacteria, the major virulence molecule is LPS. But in Gram-positive bacteria, several important virulence compounds have been identified including lipoprotein (Hashimoto *et al*., 2006) [19], peptidoglycans (Girardin *et al*., 2003) [17] and lipoteichoic acid. In contrast to *E. coli* mastitis, *S. aureus* mastitis is characterised by a moderate and delayed SCC increase, due in part, to limited cytokine response (Bannerman *et al*., 2004) [3].

4. Mastitis screening test

4.1. California mastitis test

Sargeant *et al*. (2001) [47] found that health status of udder in dairy cow was monitored by using California mastitis test (CMT). Iqbal *et al*. (2006) [22] stated CMT as the most sensitive indirect method for detection of sub-clinical mastitis in buffalo and cattle. Leach *et al*. (2008) [28] suggested that CMT was one of the best methods for detection of sub-clinical mastitis at the earliest at farm level.

4.2 Electrical conductivity test

Kitchen *et al*. (1980) [26] reported that the electrical resistance of the milk decreases when concentration of Na^+ and Cl^- ions rise in the milk in an udder suffering from intra-mammary infection. Ahmed *et al*. (2008) [1] suggested electrical conductivity test as diagnostic tool for early detection of sub-clinical mastitis in buffalo cows.

4.3 Somatic cell count

In healthy udder, majority of somatic cell count (SCC) comprised of macrophages (66-88 per cent) followed by lymphocytes (10-27 per cent), neutrophils (1-11 per cent) and epithelial cells (0-7 per cent). During mastitis, neutrophils (70 to 80 per cent) formed the major portion cell type in milk somatic cells and early influx of neutrophils resulted in early resolution of infection (Paape *et al*., 2002) [37]. Somatic cell count acted as the indirect indicator of udder health status in dairy cows.
Sharif *et al*. (2007) [51] conducted an experiment to assess the severity of sub-clinical mastitis based on SCC and they found a positive correlation between SCC and severity of sub-clinical mastitis infection in buffalo cows.
Koc and Kizilkaya (2009) [27] studied the factors which influence SCC in the milk of Holstein Friesian (HF) and Brown Swiss (BS) cows. They found that SCC varied between breed, milking time, stage of lactation and lactation number. The SCC level was the highest in the first month of lactation and the level was decreased in the later months. They suggested the possible reasons for the difference in SCC means between the breeds are due to difference in the resistance mechanisms against mastitis, morphological conformations of udders and milk yields between these two breeds.

4.4 Microbial culture

Hillerton (2000) [20] reported that detection of bacteria in milk sample was the confirmative test for presence of mastitis in cows. He also suggested that isolation of microbe was the only test with highest specificity (100 per cent) for detection of mastitis.
Sudhan and Sharma (2010) [55] stated cultural test as the gold standard test for detection of udder infection in dairy cows. Isolation of different pathogens from mastitic milk was a clear indication of infection in udder.

5. Controlling measures of bovine mastitis

Vaccination, a very innovative idea, designed specifically towards characteristic structural subunits of invading organisms, has been extensively studied in recent years. Most mastitis vaccines fail to elicit long term immune responses. Another interesting discovery demonstrating potential in the reduction of mastitis occurrence in mice and cows was lysostaphin, a bactericidal enzyme (Bramley *et al*., 1990; Oldham and Daley, 1991) [8, 34]. Kerr *et al*. (2001) [25] developed lysostaphin-transgenic mice which demonstrated

the capability of successfully preventing *S. aureus* infections. Many different controll measures of bovine mastitis include hygienic management procedures, antibiotic treatments, teat dipping sealants, and intramammary devises. However, the treatment for bovine mastitis relies heavily on antibiotic administration. The drawback of using antibiotics is that the milk may contain residuals and when not administered properly bacteria have the chance to mutate and become resistant to that particular antibiotic (Bradley, 2002) [6]. Moreover, in some cases, depending on the type of pathogens, using antibiotics alone is not sufficient to destroy the pathogen.

Along with adequate herd management and sanitary care, the selection of animals that are resistant to diseases and the incorporation of this trait in herds is a promising alternative to reduce the problems caused by infectious-contagious diseases. The use of animals that are resistant to a determined disease reduces the need to apply drugs, with consequent decrease in the levels of contamination of products and the environment. Therefore, the incorporation of genes that impart resistance through the selection of more resistant animals is a practice that should be encouraged.

One of the techniques employed to select disease resistant animals is the use of molecular markers in genetic improvement programs. Various strategies can be applied to identify these markers, among which studies of gene expression stand out. These studies generate knowledge about the biochemical and genetic mechanisms of resistance by clarifying the actions of the respective genes. In recent years, new methods to identify genes of interest by means of gene networks have become available (Chen *et al.*, 2008; Reverter and Fortes, 2013) [10, 45].

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