

From Infection to Impact: Unraveling Mastitis in Dairy Animals — A Comprehensive Review of Causes, Consequences, and Control

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Abstract

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Mastitis is well known as one of the most notable and expensive illnesses in dairy cattle and buffaloes around the globe, causing millions of losses to the economic sector and health issues among the people. Having an inflammatory nature of the mammary gland, the disease is primarily the result of pathogenic microorganisms, which are either of contagious origin (e.g., Staphylococcus aureus) or environmental origin (e.g., Escherichia coli). The pathogens gain entry through the teat canal, multiply and secrete toxins that destroy milk-secreting tissues, developing an immune response that leads to a rise in somatic cell counts (SCC). This activity has a tremendous impact on the milk composition by decreasing the contents of vital nutrients such as casein and calcium and increasing sodium and chloride content, which diminishes milk quality and processing efficiency. Subclinical mastitis is more common epidemiologically than clinical mastitis and, in many cases, it goes unnoticed and results in losses in productivity in the long-term. In other areas such as Pakistan, the prevalence of subclinical cases may be as high as 50.7 in cattle. In addition to farm economic factors,

mastitis is a public health hazard due to the possibility of zoonotic pathogen transmission and the availability of antibiotics in the milk products. Although antibiotic therapy has been a widely used treatment method, it is usually limited by

antimicrobial resistance. Modern control approaches, therefore, focus on a holistic approach of enhanced milking hygiene, application of germicidal teat dips, dry cow therapy and introducing precision dairy technologies. Resistance to prevention and improved host immunity constitute the key to sustainable management of dairy production to guarantee health of the udder, safety of the milk, and profitability of the dairy sector.

Introduction

Mastitis is widely known as one of the most significant and the most expensive illnesses that affect dairy animals, especially cattle and buffaloes, in both the developed and developing dairy production systems. It can be defined as inflammation of the mammary gland, which most of the times is caused by intramammary infection by pathogenic microorganisms and causes significant economic losses as it leads to reduced milk production, fading of milk quality, high costs of veterinary treatment and disposal of milk, early culling of animals and poor reproductive performance. Worldwide, mastitis has been posed as the most significant health condition that restricts productivity and profitability of dairy herds not just to farm economic but also to human health and safety of dairy products (1). The disease has no age and production level specificities in animals; however, the management practice, housing conditions, hygiene standards, milk process, and environmental factors have a strong impact on the disease prevalence and severity. The resulting demand to produce large quantities of milk in intensive dairy production systems has further exposed the animals to mastitis because of the physiological burden on the mammary gland, thus undermining the local immune defense. Therefore, it is evident that mastitis is still a thorn in the flesh despite major improvements in veterinary medicine, dairy management and technology in diagnosing this disease.

Mastitis etiology has a complicated and multifactorial nature, which entails a broad array of pathogenic agents, host-specific parameters, and environmental circumstances. The causative agents are mostly bacterial pathogens, which are classified into contagious and environmental pathogens (2). The infectious agents, such as *Staphylococcus aureus* and *Streptococcus agalactiae*, are mainly caused by contact during the process of milking, through use of contaminated milking equipment, towels, or hands of milkers, but environmental pathogens, such as *Escherichia coli*, *Klebsiella* spp., *Streptococcus uberis*, and *Streptococcus dysgalactiae*, are found on bedding material, manure, soil, and water. The microorganisms enter the mammary gland by way of the teat canal, especially during, or shortly after, milking, when the teat sphincter is partially open. In the mammary gland, pathogens attach to the mammary epithelial cells, proliferate and produce virulence factors, which cause an inflammatory reaction against toxins and enzymes as well as cell wall components. Infection severity is determined by virulence of pathogen, infectious dose and the host immune status (3). Mastitis can be seen as clinical disease that has observable outcomes such as swollen, painful, hot, and milk of abnormal quality, or subclinical mastitis, which does not show clinically but is characterized by an increase in somatic cell count and a great deal of loss in milk quality and processing efficiency. Subclinical mastitis is especially a thorny issue since in most cases it goes undiagnosed as it quietly leads to economic losses in the long term (4).

The pathogenesis of mastitis involves a very complex interaction between the invading pathogens and the host immune system at the cellular and molecular levels. Bovine mammary epithelial cells are not only the major point of infection but also actively involved in the innate immune protection (5). Pattern recognition receptors, including Toll-like receptors and NOD-like receptors on such cells recognize pathogen-associated molecular patterns and activate intracellular signaling pathways

and secrete inflammatory mediators, like cytokines and chemokine (6). This immune response leads to the infiltration of leukocytes, specifically neutrophils, from the blood to the mammary gland, which enlarges the count of somatic cells in the milk. Although such a response is necessary to clear the bacteria, excessive, or prolonged inflammation results in tissue damage to the milk-secreting tissues, destabilization of the blood milk barrier, and radical changes in milk composition, such as lactose, casein, calcium reduction and sodium, chloride, immunoglobulins, and serum protein increase. These alterations have a negative impact on the milk production, shelf life, and processing to the dairy products like cheese and yogurt (7). It is against the backdrop of the constraints of an antibiotic treatment mode, diminished efficacy in persistent infections, antimicrobial resistance, and the concern regarding drug residues in milk that current approaches to managing mastitis are more and more based upon prevention via better hygiene, proper milking management, dry cow therapy, early diagnosis, and host defense enhancement. Thus, the etiology, pathogenesis, and treatment of mastitis require the overall knowledge about the disease and its impact on the implementation of the sustainable control programs to enhance the health and quality of the milk as well as overall profitability of the dairy business (1).

Epidemiology of Mastitis

Mastitis is always referred as one of the most commonly occurring diseases among dairy cattle and buffaloes in the world, both clinical and sub-clinical forms have been reported to occur in different production systems. Subclinical mastitis (SCM) is more frequently observed in epidemiological surveys than clinical mastitis, as it does not manifest any symptoms, and it is more difficult to detect and manage; this asymptomatic quality enables SCM to spread over time in herds and cause an unaffected loss of economic gains. As an illustration, in bovines in a number of governorates in Egypt, mastitis pathogens like the *Escherichia coli* and *Staphylococcus aureus* have been detected in milk, teat swabs, milking equipment, and bedding indicating that the environment and management have a significant role in the occurrence of the disease. The prevalence rates differ across the regions, management, and diagnostic criteria. In a survey of dairy bovines in Egypt, the rate of *E. coli* in clinical mastitis milk was found to be 33.3, and *S. aureus* was identified in 23.1 out of clinical samples, which indicates the presence of more than one bacterial species as a common mastitis causative agent in the field. Pakistan studies have reported high levels of SCM, cross-sectional data have shown average levels of around 50.7% cattle and 46.2% buffaloes, clinical mastitis has given prevalence values of about 34.2% cattle and 28.9% buffaloes, however these are all variable with region, breed and management (8).

A study of Khyber Pakhtunkhwa province of Pakistan reported a total of 17 to 57 percent prevalence of overall clinical and subclinical mastitis, respectively, with subclinical mastitis prevailing mainly in buffaloes, as opposed to cattle (9). Such environmental factors as increased temperature and humidity are associated with increased incidence of mastitis, which illustrates the role of climatic factors in epidemiology. Other cross-sectional epidemiological studies carried out in Bangladesh have documented a prevalence of SCM at the cow level at 55.33% with some risk factors that were found to increase the rate of infection including the type of breed, age, and housing conditions. Epidemiological estimates in the world are different: meta-analysis data indicate that the prevalence rates of SCM among dairy cattle may differ greatly, between 20 and 80 percent in different countries, regardless of the diagnostic methods applied (e.g., California Mastitis Test, somatic cell counts), the herd management and the environmental conditions of hygiene. All these studies suggest that mastitis is common in dairy populations, and the regional distribution of mastitis has a considerable amount of variation that is contributed by host (age,

lactation stage, breed) and environmental (season, milking hygiene, barn hygieness) factors.

Microbes known for causing mastitis in dairy animals:

Studies have established that buffaloes are prone to mastitis disease more than cows. Some organisms have been documented to cause mastitis in buffaloes, including *Mycobacterium tuberculosis*, *Streptococci*, *Escherichia coli*, *Corynebacterium*, *Pseudomonas* spp., *Streptococcus dysgalactiae*, and *Mycoplasma* (10). Among the other pathogens of bovine mastitis, *Staphylococcus aureus* is the major organism. Various causative agents of mastitis have been identified or discovered in buffaloes as contagious pathogens or may be available in the manure, soil or in the environment around the animals as an environmental pathogen. The primary contagious pathogens are *Staphylococcus aureus* and *Streptococcus agalactiae* that are transmitted between infected and clean udders in cases of inadequate sanitization of the hands of the milker or failure to use separate cloth towels to dry the udder of each animal (11). The most important pathogen of these animals is *Staph. aureus*.

The most common environmental pathogens that can affect healthy animals when practicing unhygienic milking are *Streptococcus uberis*, *Str. dysgalactiae* and coliforms like *E. coli* and *Klebsiella* (12). Bacteria cause coliform infection and mainly when the animal is kept under unhygienic conditions. *Klebsiella* is a sort of bacteria that could be found predominantly in the animal bedding; close to 70% to 80% coliform infection presents visible indicators like abnormal milk of watery nature, hyperthermia, anorexia or an inflamed quarter (13). These are microscopic bacteria that are environmental pathogens that are likely to induce clinical mastitis, which presents visible signs and symptoms. About half of infections with environmental streptococci bacteria display visible clinical signs and symptoms. The environmental pathogens bear about 60 to 70 percent of the infections and last a short time, below one month (14). The non-lactating phase or dry period is the best period when the animal can be exposed to a new infection of environmental streptococci, and this is mostly between 7 to 14 days after milk withdrawal and 1 week to 10 days prior to parturition. The risk of occurring any type of infection around parturition or calving period is two times higher than that during non-lactation or dry period. When they get infected at the initial stages of the dry period, then they can be treated using antibiotic therapy, but when the dry period is late then they cannot be treated effectively. The effectiveness of antibiotic therapy in the environment in the dry period is about 70 percent in streptococcal infections (15). But unfortunately, in Pakistan the practice of antibiotic therapy during dry period is not implemented.

Pathogenic cascade of mastitis:

In dairy animals, mastitis is when there is inflammation or swelling of the udder epithelium, followed by the infiltration of the teat canal and the mammary gland with bacteria (16). The entering bacteria multiply and release toxins that damage the tissues of milk secretion, along with chemical irritants and physical trauma. As a consequence, the leukocytes and the number of cells in the milk is raised; consequently, it can be seen that the quality and quantity of the milk and its by-products is reduced or compromised (17). The first line of defense against infection is its orifice (the entrance of the teat canal). Externally, the teat canal is enclosed by a surround of sphincter of muscles that ensure that the canal is closed down. This is a muscle sphincter that does not just prevent milk from leaking out but also bacteria that gets into the teat is prevented. The teat canal is lined with keratin, which is made of stratified squamous epithelium, on the inside (18). It has been reported that upon damage to this keratin, it exposes the teat canal to vulnerability of bacterial invasion and colonization. This keratin has a waxy substance which is fibrous proteins and fatty acids (19). Such fatty acids can be found both in their esterified and non-

esterified forms (i.e. palmitoleic acid, myristic acid, and linolenic acid), which are bacteriostatic (they do not kill or necessarily prevent the growth and reproduction of bacteria) (20).

Fibrous proteins of the keratin in the teat canal bind electrostatically to the mastitis pathogens changing bacterial cell wall, rendering it more vulnerable to the osmotic pressure (21). Failure to sustain an osmotic pressure leads to lysis of invading pathogens. Although the arrangement of keratin allows the containment of invading bacteria, there is also a possibility of their migration into the gland cistern. Bacteria that are located near the orifice in the process of milking can get a chance of accessing the teat canal and harming the keratin or the lining of the mucous membranes of the teat sinus (22). There is always the ideal moment when the pathogens can be introduced into the teat canal, which is when the canal is incompletely closed; this can take the form of one to two hours after milking where the canal is free to allow the entry of the pathogens. The bacteria pathogens that manage to cross the orifice and evade the antibacterial measures result in the mammary gland pathogeny as the second line of defense. The mammary gland in the dairy animals comprises of the teats and the udder where bacteria proliferate and secrete toxins, enzymes and components of the cell walls (23). Upon the identification by the immune system, they cause the release of the inflammatory mediators. These body mediators trigger phagocytes- immunocytes that ingest and eliminate pathogens in the body and initiate the inflammatory process successfully.

The extent of the inflammatory response is determined by the host and pathogen factors. The species and strain, virulence, and size of bacterial infusion are considered pathogen factors whereas age, lactation stage, immune status, and somatic cell count are considered host factors (24). Mammary tissue and secretions have neutrophils in the initial phases of mastitis. After the activation process by the inflammatory mediators, the phagocytes migrate in large numbers to the invading bacteria out of the bone marrow. These neutrophils kill the bacteria using a bactericidal effect. Many anaerobic reactants, including peroxidases, are also found during phagocytosis. The neutrophils bypass the milk secreting cells to the lumen of the alveoli and lead to an increased number of somatic cells and destruction of secretory cells (25). Leukocyte deposition may cause clots, which may interfere with the blood clotting factors, resulting in blocked ducts to prevent total removal of milk (26). This may cause scar tissue due to elastic connective elements, which cause a total loss of functionality in that area of the gland. The alveoli can shrink (atrophy) and replace the smaller secretory cells, especially when the milk ducts are blocked and the secretory cells fail to work (27). As a result, tiny cavities might develop whereby antibiotics will have hard time accessing the infected area in order to eliminate all the milk.

Effect of mastitis on milk's composition:

Mastitis transacts important changes in milk as indicated in (table 1). Casein is a significant milk protein that has great nutritional value but with the elevated vascular permeability, the quality of dairy products and milk proteins like serum albumin and transferrin is deteriorated or weakened (28). Berger et al. (2008) documented that the milk proteins such as immunoglobulin and albumin are on the rise owing to the high somatic cell count; consequently, the heat stability of mastitic milk is also low and pasteurized milk scores low grades following storage (29). Also, the uptake of calcium in the blood to milk is lowered; thus, the coagulation properties of the mastitic milk are lowered or impaired. The breakdown of milk proteins occurs in dairy animals with clinical and subclinical forms of mastitis since proteolytic activity (the ability to destroy protein) is increased more than twofold in cases of infection (4, 30). Prior to the process of milk withdrawal, large-scale destruction of large quantities of casein in the udder may be caused by plasmin (a proteolytic enzyme) and the enzymes of somatic cells. The levels of chloride and sodium in the milk are on the

rise because of mastitis (31). The common principle, which is the potassium, also diminishes since casein is largely tied to calcium in milk. Reduced level of lactose is also a significant consideration to the reduced acidification level in milk that correlates with an elevated number of somatic cells (32).

Table 1: Comparison of values (%) of normal milk with that of mastitis milk having high somatic cell count

Constituent	Normal milk	Mastitis milk with high SCC	
Fat	3.5	3.2	
Lactose	4.9	4.4	
Total protein	3.61	3.56	
Total casein	2.8	2.3	
Whey protein	0.8	1.3	↑
Serum albumin	0.02	0.07	↑
Lactoferrin	0.02	0.1	↑
Immunoglobulin	0.1	0.60	↑
Sodium	0.057	0.105	↑
Chloride	0.091	0.147	↑

Integrated mastitis management and control strategies:

Mastitis cannot be eliminated totally in a flock, but the rate could be maintained to a minimum. To manage mastitis, a number of practices hold significance and they include proper sanitation and good husbandry practices. After pouring, post-milking teat dips like iodine dips may be applied to the teat to serve as a disinfectant to help sanitise the teat and prevent transmission of bacteria that cause mastitis (33). Culling of chronically infected animals and treatment of mastitis at non-lactating stage are other approaches used as critical strategies. An optimal period of the treatment of mastitis is the dry period or non-lactating period as opposed to the period during the lactation period since the medication remains in the udder longer (34). Also, the milking does not destroy the udder and there is a reduced possibility of bacterial entry into the teat canal. Close observation of the number of somatic cells and early therapy is very useful in managing the illness (35). The inverseness of the somatic cell and the udder health implies that the lower the somatic cell count the healthier has to be the udder, and the other way around. In the dry season, the antibiotics are put into the teat canal using long acting antibiotics to eliminate bacteria (36).

The main concepts of control are to decrease the exposure of teat to pathogenic bacteria or to enhance the resistance of animals to them. This entails hand washing by using soap and clean water or disinfectant and washing of the udder and teats using a sanitizing solution (37). An individual clean cloth or towel should then be used to dry the teats and the udder. Wet the teat before cleaning it with a high effective teat dip solution within a period of 30 seconds (38). One can use a cotton swab that is dipped into or rinsed with alcohol to clean the ends of the teats. When cleaning the four

quarters, you can begin with the teats that is furthest and move towards the nearest one and so on; this will ensure that the teats are washed without coming in contact with the dirty ones. Administer commercial intramammary infusion products in a single dose that is meant to be delivered as an intramammary infusion. When treating, always treat the nearest teats first and then the nearest ones then the distant one because it may contaminate clean teat ends (39). Lastly, it is necessary to dip the teats in a good germicidal teat dip when the treatment is over (38).

Staphylococcus aureus happens to be the biggest contributor of the contagious mastitis issue in dairy animals (40), which causes mastitis and poses severe issues; it is an easy-to-spread organism that is not easy-to-treat. The rate of recovery is extremely slow during the lactation period under the assistance of antibiotics (41). Majority of affected animals are untreatable, end up being chronic and finally culled. Another kind of bacteria that causes the mastitis is the *Streptococcus agalactiae* (42). The good thing with it is that it is easy to cure with the aid of antibiotics and application of effective germicidal teat dips. *Streptococcus dysgalactiae* constitutes a kind of a bacterial species that may be located in the rumen, feces, bran, as well as in the udder of dairy animals (43). This is controllable through good cleaning, sanitation or good hygienic environment and the sensitivity to antibiotics is good.

Environmental mastitis can be conquered by reduction of the number of those bacteria that contaminate the teat and penetrate through the orifice (44). The environment around the animals should be clean and dry to avoid contamination of whatever kind. Limit the ability of the animals to get access to garbage, mud, or manure disposal sites and make sure that the area where the parturition or calving occurs is clean (45). Milk then dip the teats in good bactericidal teat dips. An attempt to eliminate environmental mastitis during non-lactating phase using barrier dips, germicides, or bactericides has not been successful. All the quarters of the animal should be prescribed antibiotic treatment in the non-lactation period. It assists in management of the streptococci that exist in the environment in the onset of the dry period (46).

Public Health Significance:

Mastitis is a potentially important issue with regard to the public health because it has both a direct and an indirect impact on milk safety, quality, and potential human infection by pathogenic and antimicrobial-resistant microorganisms (47). The milk of mastitic animals contains more pathogenic bacteria including *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus* spp. and *Klebsiella* spp. some of which produce toxins or cause foodborne disease when consumed in their raw or poorly processed form (11, 12, 48). Specifically, *Staphylococcus aureus* that produces enterotoxins is a severe threat due to the heat-resistant nature of its toxins which can withstand pasteurization and cause staphylococcal food poisoning. The physicochemical properties of the milk are also affected by the effect of Mastitis such as high somatic cell count, protein and fat composition, and high enzyme activity that are harmful to milk processing, shelf life, and safety of the products (49). One of the most important health issues related to mastitis is the intensive use of antimicrobial agents as a treatment, which may lead to antibiotic residues in milk in case the adequate withdrawal intervals are not observed (50). Use of milk which has residues of antibiotics can result in allergic reactions, altering normal gut microbiota as well as interference with the dairy fermentation processes as well as assisting the development of antimicrobial resistance by human pathogens. Moreover, the presence of antimicrobial-resistant bacteria in mastitis milk may be a reservoir that can pass genes of resistance to the food chain between animals and humans (51). This is a very important issue especially in the developing nations where regulation enforcement, routine milk screening and awareness on the withdrawal time may not be up to standard. Mastitis pathogens and drug residues compromise the consumer confidence in dairy products and the national and international trade through the inability to comply with the food safety standards. That

is why the control of mastitis is not only critical to the health and dairy performance of animals but also a very important aspect of the community health protection where hygiene, responsible use of antimicrobials, regular milk testing, and food safety regulations are stressed to provide the population with a safe and high-quality milk.

Future Perspectives:

According to recent scientific literature, future interventions in bovine mastitis control and management will place more emphasis on sustainable, precision-based, and antibiotic-sparing interventions. This is due to the increasing worry regarding antimicrobial resistance and diminishing efficacies of traditional therapies in the treatment of chronic intramammary infections. Several articles that were found in Google Scholar show the urgent necessity to decrease the usage of antibiotics in favor of alternative and complementary methodology to control the situation, such as immunomodulators, probiotics, bacteriophage therapy, phyto-therapeutic compounds, or nanoparticle-based drug delivery systems, which demonstrated promising perspectives in experimental and initial field research of reducing reliance on antibiotics and improving the efficacy of the treatment. Simultaneously, molecular biology and genomics are likely to be extremely useful in the future, especially by discovering the genetic characteristics of mastitis resistance and implementing a selective breeding program that would enhance the health of the udder without affecting the milk production rate. The recent reviews also point out the growth of fast, sensitive and on-farm diagnostic methods including biosensors, molecular tests and automated somatic cell count methods will enable early identification of intramammary infections and help make targeted treatment decisions, which will reduce unnecessary antimicrobial use. Moreover, the introduction of precision dairy farming technologies, such as automated milking systems, sensor-based udder health surveillance and decision-support tools based on data, is becoming a new trend in enhancing the health surveillance and prevention of mastitis in the herd. The need to enhance antimicrobial stewardship initiatives, educate farmers, and adopt evidence-based treatment measures in reducing the onset and transmission of resistant mastitis pathogens is also a focus of the current studies and especially in developing nations. Scientifically speaking, the combined effect of scientific consensus is that the role of future mastitis control will be based on a multidisciplinary model that incorporates better diagnostics, increased host immunity, responsible antimicrobial usage, genetic selection and better management of the farm to achieve sustainable dairy production, better animal welfare, and improved safety of milk.

Conclusion:

Mastitis is among the largest problems and challenges experienced by dairy farmers in dairy farms. Mastitis leads to infection in the mammary gland of the animal and to a great extent, it influences the quality and quantity of milk and thus the milk is not suitable to be used by the human beings. Pathogenic bacterial organisms, such as *Klebsiella*, *Staphylococcus aureus*, *E. coli* and *Streptococcus agalactiae* are the major causes of mastitis. These bacteria enter the udder by way of the teat canal, principally after milking, since after milking the teat canal is left open 1 to 2 hours. After the bacteria enter the udder, it multiplies and causes toxins that destroy the milk secreting tissues. This provokes the immune system of the animal and attracts the leukocytes (white blood cells) to combat the infection; because of this, the udder swells up and the milk turns watery in its content, having clots or flakes. Once the animal contracts mastitis, the milk contains a high level of somatic cell count, thus an indication of inflammation of the udder.

Primarily, there are two kinds of mastitis the first one is clinical (that is when a sick animal presents apparent signs and symptoms), and the other type is subclinical (when no signs and symptoms are visible, however, it is still dangerous). Unhygienic

conditions such as the absence of cleanliness of the milking machine, contaminated bedding or manure cause environmental mastitis. The passing of the infection between cows is also through sharing of towels to dry off the udder or some shared equipment. Prevention is pertinent and effective in the management of mastitis compared to therapeutic management. Practice good hygienic standards, such as washing hands before and after milking, cleaning the udder or the teats with germicides or disinfectants, good teat dips, clean and dry bedding for the animals, and use an individual towel per animal. Mastitis is better treated during the dry period or non-lactation period. This is the time to administer the antibiotic treatment, which destroys the unseen bacteria that are present in the teats. The animal might also require culling once treatment is at the end, in case it is not treated as this could cause it to spread the infection to other healthy animals.

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