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Basic facts of mastitis in dairy animals

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***Abstract*— Bovine mastitis, an infection of the mammary gland, is the predominant ailment in dairy cattle, resulting in economic losses from diminished productivity and inferior milk quality. The etiological agents comprise various gram-positive and gram-negative bacteria, which may be either contagious (e.g., *Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma spp*.) or environmental (e.g., *Escherichia coli, Enterococcus spp., coagulase-negative Staphylococcus, Streptococcus uberis*). Enhancing sanitation through improved milking hygiene, post-milking teat cleaning, and the upkeep of milking machines are fundamental strategies to avert new instances of mastitis; however, the therapy of active mastitis infections primarily relies on drugs. The widespread utilization of antibiotics heightened concerns regarding the rise of antibiotic-resistant bacteria, prompting the dairy industry to diminish antibiotic usage. Consequently, alternative therapies for the prevention and treatment of bovine mastitis, especially natural materials derived from flora and fauna, have been pursued. This study offers a comprehensive examination of bovine mastitis, focusing on risk factors, management strategies, treatments, and novel therapeutic options for its control.**

***Keywords* — Bovine Mastitis, Dairy Cow, Bacteria, Antibiotic, Alternative Bovine Mastitis Treatment, Natural Products**

INTRODUCTION

Mastitis continues to be the most costly disease of dairy animals. Field surveys of major livestock diseases in Pakistan have ranked mastitis as number one disease of dairy animals (1,2). In Nili-Ravi buffaloes, mastitis shortens lactation period of each animal by 57 days on an average and reduces 438 kg of milk per lactation (1). Mastitis adversely affects the quality of milk and dairy products (3, 4). Current statistics on losses in Pakistan owing to mastitis are unavailable; nevertheless, in Punjab alone, the annual losses attributed to clinical mastitis total Rs. 240 million (5). These arise from wasted milk, decreased milk production, early culling of livestock, and substitutions. The losses attributed to clinical mastitis exclude those resulting from sub-clinical mastitis, which is less apparent and may only be identified through the measurement of somatic cell counts (SCC) in milk.

Clinical mastitis is marked by abrupt onset, edema, erythema of the udder, discomfort, and diminished or changed milk production from the affected quarters. The milk may exhibit clots, flakes, or a runny quality, followed with fever, sadness, and anorexia. Subclinical mastitis is defined by the absence of obvious indications in the udder or milk; yet, it results in decreased milk output and increased somatic cell count, with a more pronounced effect on older lactating animals compared to first-lactation heifers. A negative correlation typically exists between SCC and milk yield. Milk from healthy, uninfected quarters often contains fewer than 200,000 somatic cells per milliliter. A somatic cell count exceeding 300,000 is atypical and signifies inflammation in the udder. A substantial body of research indicates that dairy cow milk contains a physiological level of 100,000-150,000 somatic cells/ml, with elevated somatic cell counts signifying a secretory disruption(6).

Shearer and Harris (7) assert that subclinical mastitis is significant because it occurs 15 to 40 times more frequently than the clinical variant (for each clinical case, there are 15-40 subclinical instances). It typically precedes the clinical form, persists longer, is challenging to identify, negatively impacts milk quality and production, and serves as a reservoir for microorganisms that can infect other animals in the herd. Losses attributed to mastitis in Pakistan may exceed those in affluent nations, as preventive measures such as post-milking teat dipping and dry period therapy are not yet implemented in the country(8).

PATHOGENESIS

Mastitis in dairy animals arises when the udder experiences inflammation and bacteria infiltrate the teat canal and mammary glands. These bacteria proliferate and generate toxins that damage the milk-secreting tissue, in addition to physical trauma and chemical irritants. These lead to an elevation in the leukocyte count, or somatic cells, in the milk, diminishing its volume and negatively impacting the quality of milk and dairy products.

The teat orifice functions as the primary barrier against infection. A sphincter of smooth muscle encircles the teat canal externally, serving to maintain its closure (9). It also inhibits the egress of milk and the ingress of microorganisms into the teat. The teat canal is internally coated with keratin originating from

stratified squamous epithelium. Damage to keratin has been documented to enhance the teat canal's vulnerability to bacterial invasion and colonization(10). Keratin is a waxy substance consisting of fatty acids and fibrous proteins found in the teat. The fatty acids, both esterified and non-esterified, include myristic acid, palmitoleic acid, and linolenic acid, which have bacteriostatic properties (11).

The fibrous keratin proteins in the teat canal electrostatically bind to mastitis pathogens, which modify the bacterial cell wall, increasing its vulnerability to osmotic pressure. The failure to sustain osmotic pressure results in the lysis and demise of invading pathogens (11, 12). The keratin structure facilitates the entrapment of invading bacteria, so inhibiting their movement into the gland cistern (13). During milking, microorganisms located near the teat's opening can infiltrate the teat canal, resulting in stress and injury to the keratin or mucous membranes that line the teat sinus (14). The teat canal may stay partially open for 1-2 hours post-milking, during which viruses might easily infiltrate the canal(15).

Bacterial pathogens that can penetrate the teat end by evading antibacterial mechanisms initiate the illness process in the mammary gland, which serves as the host's secondary line of defense. The mammary gland in dairy animals comprises a straightforward system of teats and udder, where bacteria proliferate and generate toxins, enzymes, and cell-wall components that incite the creation of inflammatory mediators, thereby attracting phagocytes. The intensity of the inflammatory response is contingent upon both host and pathogen variables. The pathogenic factors encompass species, virulence, strain, and inoculum size of bacteria, while host factors comprise parity, lactation stage, age, immune status of the animal, and somatic cell count.

Neutrophils are the primary cells present in breast tissue and secretions during the initial phase of mastitis, comprising about 90% of the total leukocyte population. (16). The phagocytes migrate from the bone marrow to the invading germs in substantial quantities, drawn by chemical signals or chemotactic chemicals, including cytokines, complement, and prostaglandins generated by injured tissues (17,18). Neutrophils perform their bactericidal function via a respiratory burst, generating hydroxyl and oxygen radicals that eliminate germs. During phagocytosis, bacteria encounter various oxygen-independent reactants, including peroxidases, lysozymes, hydrolytic enzymes, and lactoferrin. Besides their phagocytic functions, neutrophils produce antimicrobial peptides known as defensins, which eliminate several microorganisms responsible for mastitis (19). Large quantities of neutrophils infiltrate the milk-producing cells into the alveolar lumen, hence elevating somatic cell numbers and causing damage to the secretory cells. An elevated leukocyte count in milk results in a rise in somatic cell numbers. Clots are generated by the aggregation of leukocytes and blood coagulation factors, which may obstruct ducts and hinder full milk expulsion, leading to scar development and the growth of connective tissue elements. This leads to a permanent loss of functionality in that segment of the gland. The milk ducts remain obstructed, secretory cells revert to a non-secretory condition, alveoli commence atrophy and are supplanted by fibrous tissue. This facilitates the creation of tiny pockets, hindering antibiotic penetration and obstructing the entire extraction of milk (15).

Macrophages are the primary cells present in the milk and tissue of healthy involuted and lactating mammary glands (20). Macrophages phagocytize bacteria, cellular detritus, and deposited milk constituents. The phagocytic function of macrophages can be enhanced by opsonic antibodies targeting certain microorganisms. The indiscriminate consumption of fat, casein, and milk constituents diminishes the phagocytic efficacy of mammary gland macrophages compared to blood leukocytes (21,22). Macrophages are involved in antigen processing and presentation (23). Factors contributing to mammary gland trauma include improper application of udder washes, moist teats, neglect of teat dips, inadequate preparation of milking animals or pre-milking stimulation for milk ejection, overmilking, insertion of mastitis tubes or teat cannulae, injury from infectious agents and their toxins, and physical trauma

MASTITIS CAUSING BACTERIA

Research findings have demonstrated that buffalo are equally susceptible to mastitis as cows. The etiological agents of mastitis in buffaloes include Streptococci, Staphylococci, Pseudomonas spp., Escherichia coli, Corynebacterium, Mycoplasma, Streptococcus dysgalactiae, and Mycobacterium tuberculosis. Staphylococcus aureus is the primary bacterium associated with cow mastitis (24, 25). In Pakiatan, etiological agents of mastitis in buffaloes have been reported to be Streptococcus agalactiae,*Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus dysaglactiae, Staphylococcus capotus, Streptococcus pyogenes Staphylococcus hyicus and Corynebacterium bovis* (24, 26,27,28,29).

The predominant mastitis pathogens are either present in the udder as contagious agents or in the animal's environment, including bedding and manure, as environmental agents. The most prevalent contagious pathogens are Staphylococcus aureus and Streptococcus agalactiae. Transmission occurs from infected to healthy udders during milking via contaminated hands of the milker, cloth towels utilized for washing or drying many animals, and maybe by flies. An examination of mastitis prevalence in buffaloes and cows from India and Pakistan (24) determined that infectious pathogens are primarily responsible for the majority of clinical cases, with Staphylococcus aureus being the most prevalent in both species. The predominant environmental pathogens include *Streptococcus dysgalactiae*, *Streptococcus uberis* and coliforms such as Escherichia coli and Klebsiella. Pathogen transmission may occur during milking, but predominantly occurs between milking sessions.

Coliform infections are typically linked to filthy conditions, whereas Klebsiella are present in sawdust containing bark or soil. Approximately 70-80% of Coliform infections present with inappropriate milk, udder edema, and systemic abnormalities including elevated fever, enlarged quarters, watery milk, and diminished appetite. Environmental pathogens are frequently accountable for clinical situations.

Approximately 50% of infections caused by environmental streptococci exhibit clinical manifestations. Sixty to seventy percent of environmental pathogen infections persist for fewer than thirty days and are not readily identifiable. The dry season is the phase of highest vulnerability to novel environmental streptococci infections, particularly during the initial 1-2 weeks and the final 7-10 days preceding calving or early lactation. The incidence at calving is twice that at drying out. Infections during the early dry period can be managed with dry animal antibiotic medication; however, this treatment is less successful during the late dry period. Dry period therapy can eradicate 70% of environmental streptococcal infections (15). It is unfortunate that dry period antibiotic therapy is not being practiced anywhere in Pakistan.

EFFECT ON MILK COMPOSITION

Mastitis induces significant alterations in milk (Table 1). The predominant milk protein, casein, diminishes as inferior grade whey proteins proliferate, thus impacting the quality of dairy products like cheese. Serum albumin, immunoglobulins, transferrin, and other serum proteins permeate into milk due to heightened vascular permeability. Jones (15) has indicated that elevated SCC correlate with increased amounts of serum albumin and immunoglobulins, which diminishes the heat stability of milk affected by mastitis, resulting in lower grade scores post-pasteurization and storage. Additionally, there is a reduction in calcium absorption from blood into milk, leading to compromised coagulation properties of milk affected by mastitis. Haenlein *et al.* (30) documented a notable reduction in casein content when somatic cell count in milk above 500,000/ml. The degradation of milk proteins can transpire in milk from animals exhibiting clinical or subclinical mastitis, attributable to a proteolytic activity increase exceeding twofold during mastitis. Plasmin and enzymes originating from somatic cells can inflict significant damage to casein in the udder prior to milk extraction. Mastitis elevates milk conductivity and raises salt and chloride contents. Potassium, typically the primary mineral in milk, diminishes, and since most calcium in milk is linked to casein, the disruption of casein results in reduced calcium levels in milk. The diminished lactose concentration is a significant element contributing to the compromised acidification capabilities of milk with increased somatic cell count following the addition of starter cultures (31). Jones (15) examined different constituents of normal milk with those of mastitis milk with elevated SCC, as described in Table 1.

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

Source: Jones (2006).

FACTORS AFFECTING MILK SOMATIC CELL COUNT

The assessment of milk SCC is extensively employed to evaluate udder health and milk quality. The enhanced SCC predominantly comprises leukocytes, including macrophages, lymphocytes, and neutrophils. During inflammation, the significant rise in SCC is attributed to the influx of neutrophils into the milk, with over 90% of the cells potentially being polymorphonuclear (PMN) leukocytes at this stage. Jones (15) indicated that an elevated SCC correlates with an increased risk of raw milk contamination by microorganisms and antibiotic residues. Moreover, elevated SCC heightens the suspicion that the raw milk is sourced from unsanitary conditions and ill livestock. Elevated SCC are correlated with diminished acceptability of raw milk for production and processing into consumable products for humans.

Milk from healthy, uninfected quarters often contains fewer than 200,000 somatic cells per milliliter. An SCC rise of 300,000 or above signifies udder irritation. The intensity of the SCC response manifests during the initial, acute phase of the infection, attaining its zenith within hours or days. Days, weeks, or more may be necessary for SCC to diminish following the eradication of infections. (15).

Milk from uninfected quarters exhibits minimal variation in somatic cell count as the number of lactations or days in milk increases. The somatic cell count in milk from uninfected quarters increased from 83,000 at 35 days postpartum to 160,000 by day 285. California Mastitis Test (CMT) can be utilized to assess somatic cell counts in milk samples from individual animals. The CMT reagent interacts with the genetic material of somatic cells in milk, resulting in gel formation. To get reliable findings, tests must be performed immediately prior to milking, following the stimulation of milk letdown and the disposal of foremilk. Muhammad *et al.* (32) have shown that a 3% solution of household detergent (Surf-Lever Brothers) serves as a dependable substitute for the California Mastitis Test reagent.

Jones (15) indicated that reducing the maximum permissible SCC is advantageous for milk producers and processors. Managers of herds with elevated somatic cell counts may need to implement more rigorous culling for mastitis, enhance treatments for intramammary infections, intensify measures to prevent antibiotic residues in milk, cull affected animals, incur additional expenses on facilities or milking apparatus, and refine management practices to mitigate the transmission of new infections. Consequently, attention must be directed towards appropriate milking procedures, enhanced sanitation, efficient application of teat dipping and dry period therapy, as well as the upkeep of milking apparatus. A reduced somatic cell count (SCC) should lead to increased milk production and enhanced milk quality.

PROPER TREATMENT PROCEDURES

Mastitis cannot be completely eradicated from a herd, however its incidence can be minimized. The key components in the management of mastitis encompass: effective husbandry techniques and sanitation, post-milking teat disinfection, treatment of mastitis during the non-lactating phase, and the culling of chronically diseased animals. The effectiveness of therapy during the non-lactating phase has demonstrated superiority over that attained during breastfeeding. Monitoring somatic cell counts and the timely detection and treatment of mastitis in dairy animals contribute to the reduction of mastitis. Dry animal therapy can eradicate 70% of environmental streptococcal infections. The primary premise of mastitis control is to either reduce the teat's exposure to possible pathogens or enhance the resilience of dairy animals to infection.

Jones (15) has proposed that treatment should be approached similarly to how a surgeon conducts surgery. Clean hands with soap and water, sanitize teats and udder with a disinfecting solution, meticulously dry teats and udder using separate towels, then immerse teats in a potent germicidal teat dip. Permit 30 seconds of contact time prior to removing the teat dip with a separate towel, and meticulously cleanse the teat end with a cotton swab saturated with alcohol. When treating all four quarters, begin by cleansing the teat furthest from you and progress towards the nearest teat, utilizing commercial antibiotic medicines in single-dose packages designed for intramammary infusion. Prioritize treating the teats closest to you, followed by those farther away, to avoid contaminating clean teat ends. Submerge teats in a potent germicidal teat dip following therapy.

CONTROLLING CONTAGIOUS MASTITIS

Infections caused by Staphylococcus aureus continue to be the predominant issue of mastitis in dairy animals. The cure rate with antimicrobial therapy during lactation is exceedingly low. Numerous infected animals develop chronic conditions and must be euthanized. Streptococcus agalactiae responds favorably to antibiotic treatments and can be eliminated from dairy herds through effective mastitis management measures, such as teat dipping and dry animal care. Streptococcus dysgalactiae can inhabit several environments, including the udder, rumen, dung, and barn. They can be managed with adequate cleanliness and exhibit modest susceptibility to antibiotics.

CONTROLLING ENVIRONMENTAL MASTITIS

This can be accomplished by decreasing the quantity of bacteria to which the teat end is subjected. The animal's habitat must be maintained in a clean and dry condition. The animal must be prohibited from accessing waste, muck, or stagnant water, and the calving area must be maintained in a clean condition. It is advisable to soak teats in a germicidal solution after milking. Efforts to manage environmental mastitis during the dry period, utilizing either germicidal or barrier dips, have proven ineffective. It is advisable to administer appropriate antibiotic medication to all quarters of all animals throughout the drying-off stage, as it aids in managing environmental streptococci in the early dry phase

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