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BASIC FACTS OF MASTITIS IN DAIRY ANIMALS: A REVIEW

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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 (Cady *et al*., 1983; Khan *et al*., 1991). 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 (Cady et al., 1983). In addition, mastitis impairs the quality of milk and milk products (Philpot, 2003; Ullah, 2004). Statistics of current losses in Pakistan due to mastitis are not yet available, but in Punjab alone, the total losses caused by clinical mastitis amount to Rs. 240 millions per year (Chaudhry and Khan, 1978). These occur through discarded milk, reduction in milk yield, premature culling of animals and replacements. The losses caused by clinical mastitis do not take into account those caused by sub-clinical mastitis which is less obvious and may only be detectable by measuring the milk's somatic cell counts (SCC).

Clinical mastitis is characterized by sudden onset, swelling, and redness of the udder, pain and reduced and altered milk secretion from the affected quarters. The milk may have clots, flakes or of watery in consistency and accompanied by fever, depression and anorexia. The sub clinical mastitis is characterized by having no visible signs either in the udder or in the milk, but the milk production decreases and the SCC increases, having greater impact in older lactating animals than in first lactation heifers. A negative relationship generally exists between SCC and the milk yield. Milk from normal uninfected quarters generally contain below 200,000 somatic cells /ml. A value of SCC above 300,000 is abnormal and an indication of inflammation in the udder. 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 (Hillerton, 1999).

According to Shearer and Harris (2003), sub-clinical mastitis is important due to the fact that it is 15 to 40 times more prevalent than the clinical form (for every clinical case of mastitis there will be 15-40 sub clinical cases), it usually precedes the clinical form, is of longer duration, difficult to detect, adversely affects milk quality and production and constitutes a reservoir of microorganisms that lead to infection of other animals within the herd. Losses due to mastitis may even be higher in Pakistan than developed countries because mastitis prevention practices like post milking dipping of teat and dry period therapy are not so far being carried out in Pakistan (Shakoor, 2004).

PATHOGENESIS

Mastitis in dairy animals occurs when the udder becomes inflammed and bacteria invade the teat canal and mammary glands. These bacteria multiply and produce toxins that cause injury to the milk secreting tissue, besides, physical trauma and chemical irritants. These cause increase in the number of leukocytes, or somatic cells in the milk, reducing its quantity and adversely affecting the quality of milk and milk by-products.

The teat end serves as the first line of defense against infection. From outside, a sphincter of smooth muscles surrounds the teat canal which functions to keep the teat canal closed (Murphy *et al*., 1988). It also prevents milk from escaping, and bacteria from entering into the teat. From inside, the teat canal is lined with keratin derived from stratified squamous epithelium. Damage to keratin has been reported to cause increased susceptibility of teat canal to bacterial invasion and colonization (Bramley and Dodd, 1984). The keratin is a waxy material composed of fatty acids and fibrous proteins in the teat. The fatty acids are both esterified and non-esterified, representing myristic acid, palmitoleic acid and linolinic acid which are bacteriostatic (Treece *et al*., 1966).

The fibrous proteins of keratin in the teat canal bind electrostatically to mastitis pathogens, which alter the bacterial cell wall, rendering it more susceptible to osmotic pressure. Inability to maintain osmotic pressure causes lysis and death of invading pathogens (Murphy and Stuart, 1953; Treece *et al*., 1966). The keratin structure thus enables trapping of invading bacteria and prevents their migration into the gland cistern (Habbit *et al*., 1969). During milking, bacteria present near the opening of the teat find opportunity to enter the teat canal, causing trauma and damage to the keratin or mucous membranes lining the teat sinus (Capuco *et al*., 1992). The canal of a teat may remain partially open for 1-2 hour after milking and during this period the pathogens may freely enter into the teat canal (Jones, 2006).

Bacterial pathogens which are able to traverse the opening of teat end by escaping antibacterial activities establish the disease process in the mammary gland which is the second line of defense of the host. In dairy animals, the mammary gland has a simple system consisting of teats and udder, where the bacteria multiply and produce toxins, enzymes and cell-wall components which stimulate the production of inflammatory mediators attracting phagocytes. The severity of inflammatory response, however, is dependent upon both the host and pathogen factors. The pathogen factors include the species, virulence, strain and the size of inoculum of bacteria, whereas the host factors include parity, the stage of lactation, age and immune status of the animal, as well as the somatic cell count.

Neutrophils are the predominant cells found in the mammary tissue and mammary secretions during early stage of mastitis and constitute >90% of the total leukocytes (Sordillo *et al*., 1987). The phagocytes move from the bone marrow toward the invading bacteria in large numbers attracted by chemical messengers or chemotactic agents such as cytokines, complement and prostaglandins released by damaged tissues (Persson *et al*., 1992; Banumann and Graudie, 1994). The neutrophils exert their bactericidal effect through a respiratory burst and produce hydroxyl and oxygen radicals that kill the bacteria. During phagocytosis, bacteria are also exposed to several oxygen-independent reactants such as peroxidases, lysozymes, hydrolytic enzymes and lactoferrin. In addition to their phagocytic activities, neutrophils are a source of antibacterial peptides called defensins, killing a variety of pathogens that cause mastitis (Selsted *et al*., 1993). Masses of neutrophils pass between the milk producing cells into the lumen of the alveoli, thus increasing the somatic cell counts and also damaging the secretory cells. Increased number of leukocytes in milk causes increase in the number of somatic cells. Clots are formed by aggregation of leukocytes and blood clotting factors which may block the ducts and prevent complete milk removal, resulting in scar formation with proliferation of connective tissue elements. This results in a permanent loss of function of that portion of the gland. The milk ducts remain clogged, secretory cells revert to non-producing state, alveoli begin to shrink and are replaced by scar tissue. This helps in formation of small pockets making difficult for antibiotics to reach there and also prevents complete removal of milk (Jones, 2006).

Macrophages are the predominant cells found in milk and tissue of healthy involuted and lactating mammary glands (Sordillo and Nickerson, 1988). Macrophages ingest bacteria, cellular debris and accumulated milk components. The phagocytic activity of macrophages can be increased in the presence of opsonic antibody for specific pathogens. Because of indiscriminate ingestion of fat, casein and milk components, the mammary gland macrophages are less effective at phagocytosis than are blood leukocytes (Weber *et al*., 1983; Sordillo and Babiuk, 1991). Macrophages also play a role in antigen processing and presentation (Politis *et al*., 1992). Conditions which contribute to trauma of mammary gland include: incorrect use of udder washes, wet teats and failure to use teat dips, failure to prepare milking animals or pre – milking stimulation for milk ejection, over milking, insertion of mastitis tubes or teat canulae, injury caused by infectious agents and their toxins and physical trauma.

Mastitis causing bacteria

Research finings have proved that buffalo is as susceptible to mastitis as cow (Srinivasan and Singh, 1988). The causative organisms of mastitis in buffaloes have been reported to be Staphylococci, Streptococci, *Escherichia coli,* Pseudomonas spp., Corynebacterium, Mycoplasma, *Streptococcus dysgalactia*, and *Mycobacterium tuberculosis*. Among all the pathogens of bovine mastitis, *Staphylococcus aureus* is the predominant organism (Allore, 1993; Kapur *et al*., 1992). In Pakiatan, etiological agents of mastitis in buffaloes have been reported to be *Staphylococcus aureus*, *Staphylococcus hyicus*, *Staphylococcus epidermidis*, *Staphylococcus capotus, Streptococcus dysaglactiae, Streptococcus agalactiae, Streptococcus pyogenes and Corynebacterium bovis* (Ahmed, 1966; Ghumman, 1967; Qamar, 1992; Allore, 1993; Ahmad, 2001; Akram, 2002; Khan, 2002).

The most common mastitis pathogens are found either in the udder as contagious pathogens or in the animal surroundings such as bedding and manure soil as environmental pathogens. Among the contagious pathogens, the most common are *Staphylococcus aureus* and *Streptococcus agalactiae*. These are spread from infected to clean udders during the milking process through contaminated milker's hands, cloth towels used to wash or dry udder of more than one animal and possibly by flies. Reviewing the incidence of mastitis in buffaloes and cows from India and Pakistan, Allore (1993) concluded that contagious organisms are responsible for most of the clinical cases and *Staph. aureus* is at the top of the list in both the species of animals. Among environmental pathogens, the most common bacteria are *Strepcococcus uberis*, *Str. dysgalactiae,* Coliforms such as *E. coli* and Klebsiella. Transmission of these pathogens may occur during milking but primarily between milkings.

Coliform infections are usually associated with unsanitary environment, while Klebsiella are found in sawdust that contains bark or soil. Approximately 70-80% of Coliform infections are manifested by abnormal milk, udder swelling and systemic disturbances such as high fever, swollen quarters, watery milk and depressed appetite. Environmental pathogens are most often responsible for the clinical cases. About 50% of environmental streptococci infections display clinical symptoms. Sixty to 70% environmental pathogen infections exist for less than 30 days and are not easily detected. The dry period is the time of greatest susceptibility to new environmental streptococci infections, especially the first 1-2 weeks and the last 7-10 days before calving or early lactation. The incidence at calving is twice than at drying off. Infections during early dry period are controllable by dry animal antibiotic therapy but this treatment in the late dry period is not as effective as early dry period. Dry period therapy can eliminate 70% of environmental streptococcal infections (Jones, 2006). It is unfortunate that dry period antibiotic therapy is not being practiced any where in Pakistan.

Effect on milk composition

Mastitis causes considerable changes in milk (Table 1). Casein, the major milk protein of high nutritional quality, declines and lower quality whey proteins increase which adversely affects the quality of dairy products such as cheese. Serum albumin, immunoglobulins, transferrin and other serum proteins pass into milk because of increased vascular permeability. Jones (2006) has reported that with higher SCC, the concentrations of serum albumin and immunoglobins are increased which reduces heat stability of mastitis milk and pasteurization gives lower grade scores after storage. Also there is a decrease in calcium absorption from blood into milk, resulting impaired coagulation characteristics of mastitis milk. Haenlein *et al.* (1973) reported a significant decrease in casein content when SCC in milk exceeded 500,000/ ml. The milk proteins breakdown can occur in milk from animals with clinical or sub clinical mastitis due to the presence of proteolytic activity by more than 2-fold during mastitis. Plasmin and enzymes derived from somatic cells can cause extensive damage to casein in the udder before milk removal. Mastitis increases conductivity of milk and sodium and chloride concentrations are elevated. Potassium, normally the predominant mineral in milk, declines and because most of the calcium in milk is associated with casein, the disruption of casein contributes to lowered calcium in milk. The reduced lactose concentration is one important factor for impaired acidification properties of milk with elevated SCC, after adding starter cultures (Schallibaum, 2001). Jones (2006) compared various components of normal milk with that of mastitis milk having high 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 determination of milk SCC is widely used to monitor udder health and the milk quality. The elevated SCC consist primarily of leucocytes which include macrophages, lymphocytes and neutrophils. During inflammation, major increase in SCC is because of the influx of neutrophils into milk and at this time over 90% of the cells may be PMN leukreytes. Jones (2006) reported that the higher the SCC, the greater is the risk of raw milk contamination with pathogens and antibiotic residues. Furthermore, high SCC raises the suspicion that the raw milk is produced under poor standards of hygiene and from unhealthy animals. Increased SCC are also associated with reduced suitability of raw milk for manufacturing and processing into products for human consumption.

Milk from normal uninfected quarters generally contain below 200,000 somatic cells/ml. An elevation of SCC to 300,000 and above is an indication of inflammation in the udder. The magnitude of the SCC response occurs during the early, acute stage of the infection, reaching a peak either in hours or days. Whereas days, weeks, or longer time may be required for SCC to decrease after the pathogens have been eliminated (Jones, 2006).

Milk from uninfected quarters displays little change in SCC as number of lactations or days in milk increases. Somatic cell count of milk from uninfected quarters rose from 83,000 at 35 days postpartum to 160,000 by day 285. Somatic cell counts in milk samples from individual animals can be performed using California Mastitis Test (CMT). The CMT

reagent reacts with genetic material of somatic cells present in milk to form a gel. For reliable results, tests should be conducted just before milking after stimulating milk let down and discarding the foremilk. Muhammad *et al*. (1995) have demonstrated that a 3% solution of house hold detergent (Surf-Lever Brothers) can be used as a reliable alternative of California Mastitis Test reagent.

Jones (2006) reported that lowering the maximum allowable SCC is beneficial for milk producers and processors. Managers of herds with high SCC may have to cull heavier for mastitis, increase treatments for intramammary infections, increase efforts to avoid antibiotic residues in milk and cull animals, increase cost on facilities or milking equipment, and improve management to reduce the spread of new infections. Thus, emphasis should be given on proper milking techniques, improved sanitation, effective use of teat dipping and dry period therapy and maintenance of milking equipment. Lower SCC should result in higher milk yields and better milk quality.

Proper treatment procedures

While mastitis cannot be totally eliminated from a herd, the incidence can be held to a minimum. The key elements in the control of mastitis include: sound husbandry practices and sanitation, post-milking teat dipping, treatment of mastitis during non-lactating period, and culling of chronically infected animals. The efficacy of therapy during the non-lactating period has proved to be superior to that which can be achieved during lactation. Monitoring of somatic cell counts and prompt identification and treatment of mastitis in dairy animals help in the reduction of mastitis. Dry animal therapy can eliminate 70% of environmental streptococcal infections. The fundamental principle of mastitis control is that the disease is controlled by either decreasing the exposure of the teat to potential pathogens or by increasing resistance of dairy animals to infection.

Jones (2006) has suggested to approach the treatment in the same way a surgeon approaches surgery. Wash hands with soap and water, wash teats and udder in sanitizing solution, thoroughly dry teats and udder with individual towels, dip teats in an effective germicidal teat dip. Allow 30 seconds of contact time before wiping off teat dip with an individual towel, thoroughly scrub the teat end with a cotton swab soaked in alcohol. If all four quarters are being treated, start by cleaning the teat farthest from you and work toward the closest teat, use commercial antibiotic products in single dose containers formulated for intramammary infusion. Treat teats nearest to you first, then those farthest away to prevent contaminating clean teat ends. Dip teats in an effective germicidal teat dip after treatment.

Controlling contagious mastitis

Staphylococcus aureus infections remain the largest mastitis problem of dairy animals. Cure rate with antibiotic therapy during lactation is very low. Many infected animals become chronic eases and have to be culled. *Streptococcus agalactiae* respond well to antibiotic therapy and can be eradicated from dairy herds with good mastitis control practices, including teat dipping and dry animal treatment. *Streptococcus dysgalactiae* may live almost anywhere: in the udder, rumen, and feces, and in the barn. They can be controlled with proper sanitation and are moderately susceptible to antibiotics.

Controlling environmental mastitis

This can be achieved by reducing the number of bacteria to which the teat end is exposed. The animals environment should be as clean and dry as possible. The animal should have no access to manure, mud, or pools of stagnant water and calving area must be clean. Post milking teat dipping with a germicidal dip is recommended. Attempts to control environmental mastitis during dry period, using either germicidal or barrier dips, have been unsuccessful. Proper antibiotic therapy is recommended for all quarters of all animals at drying off, it helps to control environmental streptococci during the early dry period.

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