

Mastitis- An Important Production Disease of Dairy Animals

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Some endemic diseases are implicitly associated with dairy production. These, so called, production diseases do cause large economic effects. In fact, the most expensive disease on dairy farms is mastitis, one of these production diseases. Because of the chronic nature of production diseases, economic damage is spread out over the year, and the economic damage of certain factors, such as milk production decreases, cannot directly be seen.

Mastitis (Greek, Mastos =breast + it is = inflammation) is a multietiological complex disease, which is defined as inflammation of parenchyma of mammary glands and is characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues (Radostits, *et al.*, 2000). Udder is a productive organ of dairy animals; hence for better production it should be healthy. Because of its anatomical position are subject to out side influences and are prone to both inflammatory and non inflammatory conditions.

Mastitis occurs throughout the world wherever dairy cows are found. The continuing presence of the disease may be attributed to deficient management, improper milking procedures, faulty milking equipment, inadequate housing, and breeding for ever-increasing milk yield. All of these factors are probably involved, although herd investigations often fail to incriminate specific factors. It is important to recognize that mastitis is an infectious disease and that all methods of commercial milk production may provide suitable conditions for spreading mastitis organisms from cow to cow. A considerable body of evidence has accumulated suggesting that several management and environmental factors must interact together to increase exposure of cows to mastitis organisms, reduce the cows natural resistance to disease, or aid organisms in gaining entrance through the teat canal to milk secreting tissues of the udder where they cause infection.

The occurrence of disease is an outcome of interplay between the infectious agents and management practices stressing the defense of udder. According to Kennedy and Miller (1993), mastitis is expressed by tissue injury caused by tissue invasive or toxigenic organisms, which become dominant due to upset of balance in microbial population. The recent scientific literature on mastitis is so vast that the people have concentrated on individual species of major mastitides and its various aspects of treatment and control (Kirk *et al.*, 1994; Saran, 1995). Yet the problem of mastitis remains insurmountable to the dairy farmers and poses several challenges to the modern veterinary practitioners. Today it stands second to FMD as a most challenging disease in high yielding dairy animals in India (Varshney and Mukherjee, 2002) as documentary but present scenario has been changed. As per reports of occurrence of mastitis in dairy animals, it stands at first position because prevalence of mastitis had been reported more than 90% in high yielder cross bred dairy cows (Sharma, 2003).

Sharma *et al.* (2004) reported 70.32% incidence of sub clinical mastitis in buffaloes, while Maiti *et al.* (2003) reported 70.37% incidence of sub clinical mastitis in cows. Mastitis causes heavy economic losses to the dairy industry worldwide. The first report on mastitis caused losses in India was about Rs.52.9 crore annually (Dandha and Sethi, 1962). These losses increased to Rs.6053.21 crore annually in the year 2001 (Dua, 2001). Apart from its economic importance it is also a matter of concern of carries public health significance (Vasavda, 1988). Moreover, presence of antibiotic residues in the milk is undesirable due to its public health concern. Traditionally, the mastitis control programmes are focused at use of chemical disinfectants, antiseptic or herbal teat dips (Maiti *et al.*, 2004) and antibiotic therapy. Mastitis is a complex disease and thus there is no simple solution of its control. More than 250 different microorganisms can cause mastitis. Even no single vaccine is successful to control mastitis due to its multietiological nature. However, antibiotics were introduced 50 years back for its control. But the problem in dairy animals remained as it was prior to antibiotic era. The antibiotic treatment may be help but in minimizing the losses but simultaneously may lead drug resistance. Factors such as pharmacokinetic problems, and phagocytosis depressing effect of certain antibiotics and appearance of residue in milk restricts the success of antibiotic therapy. Therefore, attention is being paid to find alternative approaches. These approaches are confined to enhance udder defense mechanism and antibacterial system in milk by using immunoregulatory micronutrients (vitamin E, vitamin C, selenium, copper and zinc), vaccines and cytokines etc. Deficiency of micronutrients may lead to serious deleterious consequences on mammary gland health and thus supplementation of micronutrients could be beneficial in control of bovine mastitis.

It is important to emphasize that the modern dairy cow is completely dependent upon man, who also the most important component of management. Since cows cannot communicate directly with us concerning deficiencies in management, environment, and hygiene, there is no substitute for keen observation by owners and herdsman to try and maintain management at a high level. Indeed, the level of management is more important in mastitis control than the specific management factors being followed. Furthermore, a positive attitude toward mastitis control is essential for success because there is no magic potion, although the perseverance and strict attention to detail will eventually yield excellent result.

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ECONOMIC IMPLICATION OF MASTITIS IN DAIRY ANIMALS

Mastitis has been and continues to be recognized as one of the major disease problems concerning the dairy industry. It is also one of the most costly diseases confronting the dairy farmer. Estimating economic losses resulting from mastitis becomes an extremely difficult task because of the many levels of infection and other factors.

Mastitis is a global problem as it adversely affects animal health, quality of milk and economics of milk production and every country including developed ones suffer huge financial losses (Sharma *et al.*, 2007).

Mastitis, the most important deadly disease of dairy animals is responsible for heavy economic losses due to reduced milk yield (up to 70%), milk discard after treatment (9%), cost of veterinary services (7%) and premature culling (14%) (Bhikane and Kawitkar, 2000). Apart from its economic importance it also carries public health significance (Vasavda, 1988).

The first comprehensive report on mastitis caused losses in India published in 1962 indicated annual losses of Rs. 52.9 crore (Dandha and Sethi, 1962). However tremendous thrust on cross breeding programme and launching of operation flood in later years resulted in tremendous increase in high yielding bovine population, leading to many fold increase in economic loss. This is evidenced from a recent report where in annual economic losses incurred by dairy industry in India on account of udder infections have been estimated about Rs.6053.21 crore. Out of this, loss of Rs. 4365.32 crore (70 % - 80 % loss) has been attributed to sub clinical version of udder infections (Dua, 2001).

Kaneene and Hurd (1990) reported the average cost of mastitis EUR 28 per cow per year and the average cost of mastitis prevention was EUR 3.56 per cow per year, varying from EUR 0 to EUR 22 in Michigan. Hillerton *et al.* (1992) calculated the cost of summer mastitis in 95 herds in England. They found that only summer mastitis, on an average, costs EUR 279 per case per year. The greatest losses that occurred were due to the loss in milk production. A loss was reported of EUR 9.03 billion per year to the UK industry due to only summer mastitis. Reinsch and Dempfle (1997) reported the average cost of treatment per case of mastitis and per cow per year EUR 20 and EUR 3, respectively.

The annual losses per cow from mastitis in the United States in 1976 were estimated to be \$117.35 and losses of milk yields caused by mastitis were 386 kg/cow per year and losses of discarded milk 62 kg/cow per year (Blosser, 1979). While these losses increased upto \$185 to \$200 per cow per year (Costello, 2004).In 1976 losses from mastitis were \$1.294 billion in U.S. and increased upto \$2 billion in the year of 2009 (Viguier, 2009).

In Canada, it is estimated that mastitis costs dairy producers \$750/cow/year in terms of lower milk production, cost of medicine, treatment time, and premature culling. Moreover, 70 to 80% of that loss is due to subclinical mastitis, which is non-symptomatic (Kirk and Bartlett,1988; Natzke, 1981).

In both clinical and subclinical mastitis there is a substantial loss in milk production. Janzen (1970) cited losses of milk per quarter per day in mastitic cows of 0.34 to 2.66 kg (9.0 to 43.3%). A recent study by Wilson *et al.* (2004) at Cornell University showed that clinical mastitis tends to strike high producing animals in second-plus lactation. In other words mastitis often hits the cows with the highest production potential, which expands the loss due to mastitis. According to the study, the estimated loss following clinical mastitis was almost 700 kg for cows in first lactation and 1,200 kg for cows in second or higher lactation (Wilson *et al.*, 2004). Rajala-Schultz reported in 1999 that cows with clinical mastitis did not return to the same production level within the remainder of the lactation, according to Miller *et al.* (2004).

Drugs necessary to treat infected animals are a direct cause of economic damage, owing to their costs. The cost of drugs varies between countries, depending on the legislation and the infrastructure of the country.

Economic damage due to discarded milk is comparable with that from decreased milk production. However, there is one difference- the discarded milk is actually reduced by the cows, which means that feeding costs for that amount of milk have to be taken into account in the calculations. The economic damage of 100 kg of discarded milk is therefore larger than for 100 kg of decreased production. Natzke (1976) estimated discarded milk losses as 27 kg/day for 5 days for each clinical case and 1 to 1.5 clinical cases/cow per year in herds without teat dip and dry cow therapy programs. Dobbins (1967) estimated the losses from discarded milk as 34 kg or \$7.72/cow per year in 31 random herds involved in the Georgia Quality Milk Program.

A study by Kossaibati and Esslemont (2000) in UK reported that, on average, 10% of cows with mild mastitis are culled. In severe cases the risk of culling is assumed to be at least 20%. A cull cow is assumed to cost £420 per cull, and the cost of a fatality is £1251. Prices of all items involved are based on February 2000 values. A recent study showed (table 1) a details economic losses due to mastitis.

Table 1. Estimated annual losses¹ due to mastitis in \$. Figures from Current Concepts in Bovine Mastitis, National Mastitis Council, 1996.

Source of loss	Loss per cow (\$)	Per cent of total (%)
Reduced milk yield	121.00	66.0
Discarded milk	10.45	5.7
Replacement cost	41.73	22.6
Extra labour	1.14	0.1
Treatment	7.36	4.1
Veterinary services	2.27	1.5
TOTAL	184.40	100.0

These estimates are important in determining cost-effective measures for mastitis control and prevention strategies.

ETIOLOGY

Mastitis etiology/bacteriology provides relevant information for the farmer and veterinarian to inform mastitis treatment and prevention strategies and should be carried out as a routine irrespective of mastitis incidence and bulk milk quality.

FARM MANAGEMENT & DISEASES

Selecting the correct cows and quarters to sample, the correct number of samples, sampling technique and result interpretation are important to ensure an accurate assessment of the causes of mastitis in a herd.

Mastitis is an inflammation of the milk secreting tissue of the udder caused by bacterial infections. Today's, mastitis is considered to be a multifactorial disease. Whilst over 200 microbial species, sub species and serotypes have been isolated from bovine mammary gland (Mallikarjunaswamy and Krishnamurthy, 1997) and identified as causative agents of mastitis. Apart of different species of bacteria, several other groups of micro-organisms such as virus, fungi, yeast, algae and chlamydia can cause mastitis in cattle and buffeloes.

Mastitis is caused by many bacteria, which include the coliform group (specifically *E. coli*, Enterobacter, *Klebsiella* spp. etc.), Streptococci, Staphylococci, Coerynebacteria, Pasteurella, Mycoplasma, Leptospira, Yersinia, Mycobacteria, Pseudomonas, Serratia etc. In India, Staphylococcus, Streptococcus and *E.coli* generally cause 90-95% of all infections of mammary gland (mastitis). The goal of every dairy farmer should be to minimize the number of organisms permitted to come into contact with the teats. Fortunately, the vast majority of mastitis cases is caused by a relatively small number of microorganisms that can be grouped into three categories: (1) contagious; (2) environmental; and (3) other.

Contagious: The important organisms of this group are-

- *Staphylococcus aureus*
- *Streptococcus agalactiae*.
- *Corynebacterium bovis*
- *Mycoplasma* species.

Many workers from India have been reported that *Staphylococcus* spp. is the chief etiological agent of mastitis in cattle and buffaloes (Sharma *et al.*, 2007; Sharma, 2008; Singh *et al.*, 2005; Sharma *et al.*, 2007). The organism is ubiquitous and can colonize the skin as well as the udder. Antibiotic treatment is not always successful for certain isolates. *Staphylococcus aureus* is capable of causing peracute, acute, subacute, chronic, gangrenous and subclinical types of mastitis. The acute form of the disease usually occurs shortly after parturition and tends to produce gangrene of the affected quarters with high mortality. Grossly, the affected tissues are swollen, tense, hot, firm and painful. Milk secretion is reduced. Gangrenous tissues become blue and eventually black in color.

Streptococcus agalactiae was a major cause of chronic mastitis in pre-antibiotic era and is still a serious cause of chronic mastitis in some herds, although it can be eradicated readily by proper antibiotic therapy and management. *Strep. agalactiae* multiplies in the milk and on the mammary epithelial surfaces, generally causing a subacute or chronic inflammatory reaction with periodic acute flareups. The affected tissue eventually is destroyed resulting in reduced milk production or agalactia.

Environmental:

- *Escherichia coli*
- *Klebsiella pneumoniae*
- *Klebsiella oxytoca*
- *Serratia* species
- *Citrobacter* species
- *Enterobacter aerogenes*
- *Streptococcus uberis*
- *Streptococcus bovis*
- *Streptococcus dysgalactiae*

Other organisms:

The vast majority of organisms in this group rarely cause clinical mastitis and are not of serious economic importance to the dairy industry, though they do infrequently cause serious problems in dairy herds that do not practice good management including-

- Coagulase-negative staphylococci (CNS)
- *Serratia* species
- *Pseudomonas aeruginosa*
- *Nocardia asteroides*
- *Prototheca* species
- *Candida* species (yeasts)
- *Arcanobacterium pyogenes* (*Corynebacterium pyogenes*).

Coagulase-negative staphylococci have traditionally been considered to be minor mastitis pathogens, especially in comparison with major pathogens such as *Staphylococcus aureus*, streptococci and coliforms. The main reason for this is that mastitis caused by CNS is very mild, and usually remains subclinical (Taponen *et al.*, 2006). The significance of CNS, however, needs to be reconsidered as in many countries they have become the most common mastitis-causing agents (Pitkala *et al.*, 2004; Tenhagen *et al.*, 2006). There is no doubt that some CNS species should be considered as mastitis pathogens but the large number of species included in the CNS group blurs our current understanding of their role in mastitis. Despite intensive aetiological research, still around 20-35% of clinical cases of bovine mastitis have an unknown etiology.

FARM MANAGEMENT & DISEASES

Source of infection/Transmission

The transmission of mastitis is depends on the type of organism involved whether contagious or environmental.

Contagious organisms-

Spread of contagious pathogens from an infected cow to an uninfected cow occurs almost exclusively during the milking process. Purchased herd replacements are sometimes the source of contagious organisms not previously seen in a dairy herd.

Some general recommendations for controlling contagious mastitis organisms include:

- (1) Milking teats that are clean and dry
- (2) Using good milking machines correctly
- (3) Dipping teats after every milking (teat spraying is almost never as effective as teat dipping)
- (4) Treating quarters infected with *Streptococcus agalactiae* during lactation
- (5) Treating every quarter at drying off with a specially formulated antibiotic
- (6) Segregate known infected cows if possible, and
- (7) Prevent heifers from becoming infected by practicing excellent fly control and possibly treating heifers at or near parturition. Some animals with chronic infections may need to be culled to prevent them from being a reservoir of infection for other animals in the herd.

Environmental organisms-

The most common sources of environmental organisms include- bedding materials, manure, dirt and mud, pools of standing water and feeds. The most important single source is bedding materials because teats are in frequent and prolonged contact with bedding. Thus, prevention of teat contamination is critically important and the practice of maintaining bedding materials in a dry condition will aid in minimizing populations of these organisms. Experience has also shown that areas around shade areas can harbor populations of environmental organisms comparable to those found in the bedding of housed animals.

Other sources of these organisms include- contaminated rags and sponges used for washing udders, wet and manure-covered alleyways, multiple dose containers of antibiotics, areas around feed bunks and contaminated treatment syringes, cannulas, and needles.

For controlling the environmental organisms, the primary emphasis must be placed on decreasing moisture in the environment and decreasing exposure of teats to potential environmental pathogens during both lactating and dry periods. Exposure of teats to the organisms can be reduced by:

- (1) Maintaining cows on clean pastures (shade areas must also be clean).
- (2) Using inorganic bedding such as sand in free stalls that are well maintained to minimize moisture content.
- (3) Maintaining dry cows and heifers in the cleanest possible environment on the dairy farm, especially during the 2 weeks prior to calving.
- (4) Using excellent premilking hygiene, which includes predipping and the application of milking units to teats that are clean and dry.

Other important control methods include- treatment of every quarter of every cow at drying off, feeding all animals diets that contain adequate amounts of vitamins A and E and the trace mineral selenium, vaccinating cows with a mutant strain of *E. coli* J5 vaccine for the control of Gram-negative infections, using properly functioning milking equipment in the correct manner and reducing stress on animals to the practical minimum.

Other organisms-

Coagulase-negative staphylococci (CNS) are isolated more frequently from milk samples than any other organism. They are often referred to as "**skin flora opportunists**" because they can be isolated from teat skin, teat canals, vaginas, hair coats and nasal passages. They are neither contagious nor environmental and they rarely cause clinical mastitis. Even when they do cause clinical cases the cases are usually very mild.

Serratia organisms are of environmental origin and cause mastitis in both lactating and dry cows. They are frequently isolated from soil, water, feed, and grass. Thus, the organisms come into contact with teats from environmental sources. The infections do not respond well to antibiotic therapy. *Serratia marcescens* has been isolated from certain teat dips and teat dip containers, especially chlorohexidine dips.

When the problem of *Pseudomonas aeruginosa* then all water sources should be tested immediately. The organism has been isolated from hot water heaters that provide warm water for washing teats and udders. In addition, the organism has been isolated from wet bedding, improperly cleaned milking equipment, wet areas in the environment, soil, feces, contaminated antibiotic preparations and syringes and a few non-iodophor teat dips.

Traditional mastitis control methods that include teat dipping and dry cow treatment are effective in controlling the CNS, but the degree of control is less than for contagious organisms. These organisms account for 60% of all infections at first parturition, but the spontaneous cure rate in early lactation is often as high as 40%. The infections also occur during the dry period but many disappear during the first few weeks after calving. Control of Serratia organisms is best achieved by keeping cows in as sanitary an environment as possible to minimize exposure of teats. The practice of predipping with an effective teat dip, followed by careful drying of the teats before attaching milking units, may also aid in reducing the number of contaminating organisms on teat skin. Use of the J5 mutant Gram-negative core antigen vaccine will also aid in reducing the incidence and severity of clinical cases. Elimination of *Pseudomonas aeruginosa* infections involves culling of affected cows; or destruction of mammary gland tissues by having the herd veterinarian inject an appropriate chemical.

RISK FACTORS

The timing of mastitis outbreaks often gives important clues to the origin of herd problems. Infection rates for environmental streptococci are highest before calving, during early lactation, and near dry-off. The beginning and end of the dry period are high-risk times for the development of IMI with these organisms. During the early weeks of the dry period, the udder is many times more susceptible to infection than during the preceding lactation. Cows with environmental pathogens isolated at dry-off were **4.5 times** more likely to have a new clinical case in their next lactation (Bradley and Green, 1999). To simplify understanding of the mastitis complexity, it is useful to consider risk factors or disease determinants, which include- host (cow or buffalo) characteristics, infectious micro-organisms, environment, milking practices, housing practices, nutrition etc.

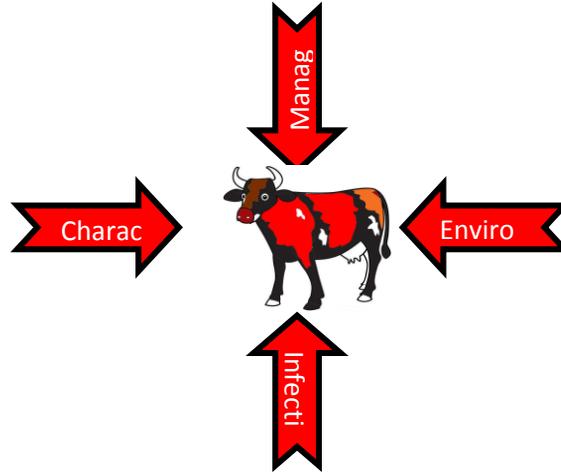


Fig. 1. Mastitis is the outcome of number of interacting risk factors.

Age and parity

Increasing parity increased the risk of clinical mastitis in cows and buffaloes (Sharma, 2003; Kumar and Sharma, 2002; Sharma and Prasad, 2002; Whist *et al.*, 2006; Kavitha *et al.*, 2009), although the reason for this association is not clear. Sharma *et al.* (2007) conducted a study on 500 lactating buffaloes of different age, parities and stage of lactation belonging to different organized or un-organized dairy farms, Chhattisgarh State, India and found that the higher prevalence of subclinical mastitis (SCM) in buffaloes was recorded in 5 to 9 years old animals and in 3rd and 4th parities. Older cows (>10 years) are at more risk (44.6%), particularly for subclinical mastitis (38.6%), than younger cows (23.6%) in which clinical mastitis was predominant (Biffa *et al.*, 2005). Cows with many calves (>7) have about **13-times** greater risk (62.9%) of developing an udder infection than those with fewer (≤3) calves (11.3%) (Biffa *et al.* 2005).

Breed and milk yield

Risk of mastitis varies from breed to breed. High yielding cows are generally considered to be more susceptible to intramammary infection e.g. Holstein Frisian (HF), Jersey or HF and Jersey cross bred dairy cows are more susceptible to mastitis than Desi (Zebu) breeds of cows (Sharma, 2003) it might be due to more resistance to disease and they are low milk producer than cross bred cows. Increased risk of clinical mastitis in Friesian compared with Jersey and Ayrshire heifers (Compton *et al.*, 2007).

Stage of lactation

The lot of work has been done on this aspect in India and other countries. The incidence of mastitis is higher during just after parturition (first 2 months of lactation) and first 2-3 weeks of dry period and Corbett (2009) suggests that the highest number of clinical mastitis cases occurs during the first week of lactation, and that the lactating cow is more likely to develop clinical mastitis during the first three months of lactation than the remainder of the lactating period. Risk of new environmental *Streptococcal* infection is influenced by stage of lactation, parity, nutrition, and immunity, in addition to factors that increase teat end exposure.

Milking techniques

It has been suggested that overmilking may increase the risk of intramammary infection. Different types of milking methods (e.g. Stripping, knuckling, full hand method, machine milking etc.) are practiced by dairy farmers to milk out the animal in India. Accurate milking practices do not cause any harm to tissue parenchyma of the udder while faulty milking practices especially knuckling causes great harm to tissue and this becomes prone to infection. The milking procedure is one of the most important risk factors for both clinical mastitis and high somatic cell count. Malfunctioning machine milking, knuckling and stripping method of milking can induce damage to the teat tissue increasing the risk for intramammary infections.

Milking machine

As per reports prevalence of mastitis is high in machine milking cows than hand milking. There is unequivocal evidence that the events occurring at milking time influence the incidence of mastitis. These influence may be via the hygiene practiced at milking time or because of effects of the milking machine *per se*. The milk secreted by infected cows

FARM MANAGEMENT & DISEASES

contains varying numbers of pathogenic micro-organisms. Herd milking provides opportunities for the transmission of these organisms between quarters and cows via the milking machine itself, milker's hands or cloths.

Milking interval

The influence of an irregular interval between morning and evening milking (<12 or >12 h/day) on the prevalence of mastitis may have been the consequence of an enhanced chance for bacteria to colonize teat ends and streak canals during the longer milking intervals and results mastitis. Hence, it is suggested that milking should be done at fixed time interval to reduce the incidence of mastitis.

Milking hygiene

Moisture, mud, and manure present in the environment of the cow are the primary sources of exposure for environmental mastitis pathogens, and hygiene scores of cows provide visible evidence of exposure to these potential sources. Milking hygiene reduce the pathogenic organisms from inhabiting the immediate environment or skin of the animals and minimizing their spread during milking process. The practice of regular teat dipping is not much more common at house hold level in India. Therefore, prevalence of mastitis in cows and buffaloes is more at unorganized dairy farms as compared to organized dairy farms. Udder hygiene significantly associated with the risk of environmental pathogen intramammary infection in cows (Compton *et al.*, 2007).

Udder immunity

The primary physical defense for the mammary gland is the teat duct. The teat duct represents the portal of entry for the majority of mastitis pathogens and represents the first barrier to infection. At the end of the teat is surrounded by sphincter composed of circular smooth muscle fibers which remains constricted and prevent leakage of milk until milking commence and entry of the organisms into the teat canal. The sphincter muscle surrounding the teat duct is tightly closed between milkings and impedes bacterial intrusion from the teat opening into the interior of the gland. It is suggested that teat orifice is remain open upto 2 hours after milking. The teat canal contains keratin which occludes the teat duct, and impedes movement of bacteria through the teat duct. When the integrity of the teat canal is damaged, the quarter will be predisposed to intramammary infection. The cellular defense of the mammary gland is the major immunologic defense of the mammary gland. During active mastitis, neutrophils represent the primary cellular defense against invading pathogens. The presence of other substances such as lactoferrin, immunoglobins, lysozymes and lactoperoxidase inhibit the proliferation of pathogens within the udder.

Udder and Teat injury

Changes to teat tissue, particularly the skin of the barrel, teat-end, and teat canal may favor penetration of bacteria into the udder and increase the risk of new mastitis infections (Hamann *et al.*, 1994).

Season

The occurrence of mastitis varies from season to season, because growth and multiplication of organisms depends on the specific temperature and humidity. The incorrect ventilation and high temperature and relative humidity enhance the multiplication of various bacteria on the skin. Exposure of animals to high temperature can increase the stress of the animal and alter the immune functions and udder health and increase the susceptibility to intramammary infection. Healthy teat skin is coated with a protective mantle of fatty acids that slow the growth of bacterial pathogens. In India, the prevalence mastitis is high in summer and rainy months and less in winter months.

Housing system

Housing facilities and management practices on farms contribute to the contamination of environments and the exposure of teats to the environmental pathogens. Poorly designed facilities can contribute to increased incidence of environmental mastitis. In all housing systems, high stocking density, dirty bedding or ground, infected utensils, poor ventilation and high humidity are important risk factors. Housing increases the risk of mastitis because the confinement of the animals and the multiplication of micro-organisms in the various litters elevate teat challenge, and consequently mastitis. The relationship between housing and mastitis is most clearly established for coliform mastitis.

Dietary factors

Work of the past 10 years has clearly demonstrated that diets of dairy cows can influence the resistance of cows to intramammary infection. Specific components of diets that have been shown to be important are vitamins E, A, and β -carotene and the trace minerals selenium, copper, and zinc. Evidence clearly shows that vitamin E and selenium influence phagocytic cell function, and cows fed diets deficient in either component are at greater risk of environmental streptococcal mastitis (Smith *et al.*, 1984). Vitamin E and selenium improved udder health, and the effect of dietary supplementation is most evident at calving and in early lactation (Sharma and Maiti, 2005). Similarly, deficiencies in vitamin A and β -carotene are associated with increased incidence of mastitis.

STATUS OF MASTITIS IN DAIRY ANIMALS IN INDIA

In India, the teat dipping as a preventive measure is not regularly practiced by dairy farmers; hence, it is essential to educate the farmers regarding the risk factors of mastitis and also about teat dipping (Kavitha *et al.*, 2009). Surveys of the prevalence of mastitis in most countries, irrespective of the cause, show a comparable figure of 50% among dairy cows and a quarter infection rate of 25% (Radostits *et al.*, 2000). Subclinical mastitis is believed to be more prevalent than clinical mastitis in most countries. The prevalence of subclinical mastitis on farms could range from 19 to 78% (Tuteja *et al.*, 1993). Sharma *et al.* (2006) had been reported 36.69% and 16.78% prevalence of mastitis at cow and quarter level, respectively in subclinical mastitis affected cross bred cows (Sahiwal and Jersey) by cultural examination from Haryana. A study from Rajasthan showed 60.25% prevalence in cows and 39.00% in quarters by cultural examination, and highest prevalence was found in 6th lactation on quarter basis and 3rd lactation on animal basis (Chahar *et al.*, 2005). De and Mukharjee (2009) have been reported the overall prevalence of clinical mastitis and subclinical mastitis were 15.18% and 42.93% respectively during the month of July and August in Uttar Pradesh. Nauriyal (1996) reported overall incidence of clinical and subclinical mastitis in cows to be 74.56% and 74.10% respectively, in

FARM MANAGEMENT & DISEASES

Uttaranchal. Das and Joseph (2005) collected 102 milk samples from clinical cases of mastitis in buffalo from Jabalpur region of Madhya Pradesh and found 84.31% prevalence of mastitis by cultural examination. Sharma (2003) had been reported prevalence of subclinical mastitis in cows and buffaloes 78.54% and 68.60% respectively, by MCMT in Chhattisgarh State. Sharma (2009) had been reported prevalence of subclinical and clinical mastitis 42.18% and 10.93% respectively in dairy cows in Jammu. A study in Jammu by Sudhan *et al.* (2005) suggests that *Staphylococcus aureus* (56.89%) is major pathogen followed by *Micrococcus* spp. (15.51%), *Bacillus cereus* (12.06%), *Staphylococcus epidermidis* (8.62%), *Klebsiella* spp. (3.44%), *Escherichia coli* (1.72%), and *Corynebacterium* spp. (1.72%). Sharma *et al.* (2007) also isolated *Staphylococcus aureus* and *E. coli* from acute clinical mastitis in buffalo.

CLINICAL SIGNS

The mastitis may be classified into subclinical and clinical Mastitis. Subclinical mastitis is a form of mastitis in which there is no readily detectable change in the udder itself and no observable abnormality of the milk. Clinical mastitis indicates that there are visible changes in the udder, such as swelling, heat, redness, pain and disturbed functions, and or visible changes in the milk, such as clots (gargot) or watery secretions and systemic reactions in varying degrees. Clinical mastitis further divided into peracute, acute, subacute and chronic mastitis.

Peracute- In which hot, swollen, redness and painful quarter/udder and sometimes gangrenous changes and abnormal milk (reduction in quantity, thin and watery sometimes blood stained) from the gland are accompanied by fever (104-106°F) and other systemic disturbances such as marked depression, shivering, rapid and weak pulse, weakness, complete anorexia and toxemia. In cases where therapy is delayed or improper recumbency and/or death may occur in a few hours.

Acute- In this form changes in the gland are similar to those in peracute but fever (103-105°F) and depression is slightly moderate i.e. intensity of systemic signs is less as compared to peracute.

Subacute- In this form no systemic changes and the changes in the gland and milk are less marked than acute mastitis.

Chronic- In this type no systemic reactions and hardness of udder/quarter may or may not be present, sometimes fibrosis and yellow coloured /watery milk with flakes or custard like. An inflammatory process that persists over many months or from one lactation period to the next is called chronic mastitis. This type exists for the most part in a subclinical form with periodic flare-ups producing sub acute or acute clinical signs which commonly subside shortly thereafter, reverting to the subclinical form. Grossly, the affected tissue is tough and smaller than normal (due to proliferation of fibrous connective tissue and glandular atrophy).

Subclinical- In this form inflammatory reaction within the mammary gland is detectable only by indirect tests and cultural isolation or the existence of inflammation in the absence of gross signs is referred to as subclinical mastitis.

The existence of a pathogen within the mammary gland without evidence of mastitis is called a *latent* infection. *Suppurative mastitis* is usually associated with *Corynebacterium pyogenes* or *Pseudomonas aeruginosa* infection. Multiple abscesses are present. While *Granulomatous mastitis* is occurs in the response to chronic inflammation by a variety of organisms like Mycobacterium, Nocardia, Cryptococcus, etc.

Detection of subclinical and chronic mastitis and of latent infection may require repeated determination of milk constituents and culturing of milk, particularly of incubated milk samples. Diagnosis of mastitis or infection in the absence of gross clinical signs generally is based on laboratory findings.

DIAGNOSIS

Unlike the clinical mastitis, in sub clinical mastitis there is neither visual abnormalities in milk (like blood, clots, flakes etc) nor in mammary gland (like swelling, hotness, cracks etc). Therefore, knowledge of routine physical examination of udder and diagnostic screening tests for early detection (i.e. during sub clinical form) of mastitis and proper treatment of affected animal is one of the paramount importance in order to minimized losses encountered due to sub clinical as well as clinical mastitis. Clinical mastitis is easily visible/diagnosed as udder swelling, pain and drastic decreased milk production even by farmers also.

Physical examination of udder

The clinician's initial step in the detection of bovine mastitis is a careful physical examination of the udder. A physical examination may be defined as clinical examination of the mammary glands by visual observation and digital palpation. The digital palpation is determined by employing the fingers and thumbs of both hands. Examine the teat orifice by turning the ends of the teats upward for a clear view. By gentle pressure note the ease of milking. To examine the teat duct and teat cistern, grasp the end of the teat with the fingers of the left hand, and pull down gently to stretch the teat. Then with the thumb and forefinger of the right hand examine the canal and cistern from the distal end (teat orifice) to the base of the udder. Abnormal lobulation or contour can see by this method. The results of the physical examination, when correlated with other observations, facilitate the clinician for a complete diagnosis. Physical examination of each gland must be made on the empty udder. The most opportune time, therefore, is immediately after milking when the hormone stimulation has ceased and the udder is completely relaxed (Sharma *et al.*, 2009).

By visual observation or inspection clinician can observe so many undesirable features like udder symmetry (e.g. oat like, rounded, step shaped and pendulous udder) and, teat shape and placement which can help in the confirmatory diagnosis. The age of the animals should be kept in mind by clinician or examiner. The size of the udder often increases gradually with each lactation, becoming heavier and more pendulant with age and production. With the use of the physical examination, it is possible to select the udders most suitable for specific therapy and to identify the cows in which the disease has advanced to such extent that slaughter is indicated. A careful physical examination is essential in any complete mastitis control programme and can not be ignored even though it does not provide the bacteriological information necessary for treatment and for prosecution of a complete mastitis-control programme based on bacteriological methods.

Milk examination

FARM MANAGEMENT & DISEASES

Before conduction of any mastitis test, the freshly drawn milk should be examined by necked for the visible abnormalities in the milk. In dry period milk changes to watery. Appearance of udder secretion in advanced cases of **chronic mastitis** is usually abnormal in appearance at irregular intervals. In case **acute mastitis**, the secretion becomes grossly altered in the early stages of the disease. The visible abnormalities may be the presence of flakes or clots in the milk, or the milk may be thin or watery and at times yellow in color (Sharma *et al.*, 2009).

Over a period of years many tests have been developed for the diagnosis of mastitis. For convenience they may be divided into two groups, viz., **Direct or cultural tests**- to determine the presence and identity of mastitis organisms in the milk and, **Indirect Tests**- which depend upon the development of palpable lesions in the udder or changes in the composition of milk;

Direct/Cultural test:-

The gold standard for determining udder infection status is milk culturing. Finding mastitis pathogens in milk is a clear indication of potential problems especially with certain bacterial species. However isolating no pathogens from clinical samples is common and rather confusing. But time consuming test and required technical skill and laboratory facilities, while most of indirect tests are easy, quick and can be performed by dairy farmers in the field. For cultural examination collection of milk sample is more important aspect to prevent false results or contamination. A key for success is a clean milk sample that is properly stored and handled from the farm to the laboratory. Failure in any of these areas can lead to meaningless information.

Collection of milk sample-

Strict aseptic procedures must be used when collecting milk samples in order to prevent contamination with the many microorganisms present on the skin of cow's flanks, udder and teats, on the hands of the sampler, and in the barn environment. The following procedures help to reduce contamination during sample collection.

- Always take a sample prior to administering an antibiotic treatment.
- Ideally, the udder should be dry and free of visible organic material.
- Clean and disinfect the teat ends with 70% ethyl alcohol or Lugol's solution and remove visible organic material from external orifice of the streak canal.
- Sterile glass or disposable plastic vials with tight fitting screw caps are to be used. Vials of at least 15 ml capacity are usually most convenient to handle, but smaller vials may be used.
- To reduce contamination of the teat ends during sample collection, sample the near teats first, then the far ones. Remove the cap from the sample vial, and without touching its inner surface, hold the cap so that the inner surface faces down. Hold the vial as near the horizontal as possible, and by turning the teat to a near horizontal position, direct streams of milk into the vial. Do not allow the cap or the vial opening to touch the teat end.
- A sample size of 3 to 4 ml is usually adequate for cultural isolation.
- To minimize the opportunity for contamination, collect each sample and replace the cap as quickly as possible.
- After collection, milk sample should be transported to laboratory in ice-box and stored at 4°C till further analysis.

Streaking and identification-

Isolation and identification of bacteria is done on the basis of morphological, cultural and biochemical characteristics. Briefly, 0.01ml of milk is streaked vertically across the diameter of an agar plate. The inoculum is then evenly spread over the entire surface of the plate by a back and forth motion at right angles to the central streak, using the same loop that was used for inoculation. Milk samples are plated on MacConkey agar to detect coliforms and gram-negative bacteria, modified Edward media for streptococci and streptococci-like organisms, Vogel-Johnson agar for staphylococci, and modified Hayflick medium for Mycoplasma organisms. Plates are incubated at 37°C for 48 hours. Mycoplasma medium is incubated under modified atmospheric conditions. The plates were observed for bacterial growth after an incubation period of 24 and 48 hours. Bacterial colonies should be subjected to Gram's stain for identification of Gram negative and Gram positive micro-organisms. After tentative confirmation of type organisms, then subjected to different and specific biochemical tests for confirmation of species of micro-organisms.



Metallic sheen of *E. coli* on EMB agar.



Colonies of *E. coli* on McConkey agar

Indirect tests:-

Indirect tests are useful in determining the quality of milk, and in the absence of laboratory facilities those which are suitable for use in the field conditions may be helpful in detecting and eliminating some of the cows that are affected with chronic mastitis. Certain indirect tests, especially the leukocyte count, are needed to supplement cultural findings in the diagnosis of mastitis (Sharma *et al.*, 2009).

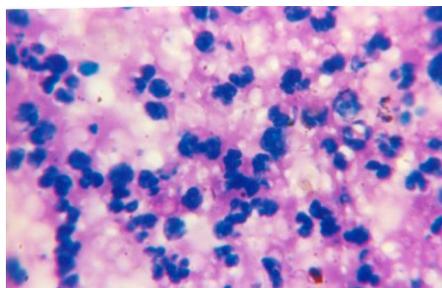
1. Somatic cell count-

Somatic cells are always present in milk and they increase due to mammary gland infections. When udders are healthy the somatic cell count (SCC) in milk is between 50,000 and 100,000 cells/ml (Skrzypek *et al.*, 2004). If the SCC is greater than 200,000 cells/ml, it is assumed to be a threshold distinguishing a healthy udder from a diseased udder (Skrzypek *et al.*, 2004; Harmon, 2001). High SCC in milk reduces the quality of both milk and dairy products, and also affects milk shelf life and flavor, as well as cheese and butterfat yield. The leukocyte count in the mastitis milk was performed to assess the degree of infection in the respective quarter(s).

Procedure

The test milk samples were thoroughly mixed by gentle shaking the vials and 10µl (0.01ml) of milk was taken on the pre drawn 1 cm² marked area over a grease free clean glass slide which was uniformly smeared with a standard sterilized bacteriological platinum loop. The smear is dried and stained with the modified Newman’s Lampert stain, by keeping the prepared slide in the staining solution for 1 to 2 minutes. The smears were gently washed in tap water and dried. The dried stained smears were examined under the oil immersion lens of the microscope.

The counting of cells in 30-50 different fields under oil immersion lens (100x) and calculate average number of the cells per field by dividing total number of cells by number of fields counted. The average number of cells per field multiplied by the multiplication factor of the microscope i.e. 497512 to obtain the number of cells per ml of the milk.



Somatic cells in milk smear under oil immersion (100X)

2. California Mastitis Test-

Estimating somatic cell concentration using the CMT test has been a valuable tool for cow-side evaluation. The CMT is simple, economical, fast and easy to use as a cow-side test. While the cell count range of each category is quite large it still is a good tool. It provides no indication of bacteria type but the score can be used to determine infection status of individual quarters. The original Schalm reagent (Tri-ethanolamine sulphate and bromocresol purple) is not available in India. But recently B.V. Biologicals, India launched a CMT reagent along with plastic paddle by the name of CMT kit. The accuracy of this method is found to be 88.66% (Sharma, 2003). Fresh, unrefrigerated milk can be tested using the CMT for upto 12 hours, reliable readings can be obtained from refrigerated milk for up to 36 hours. If stored milk is used, the milk sample must be thoroughly mixed prior to testing because somatic cells tend to segregate with the milk fat. The detail procedure is as per Sharma *et al.* (2009)-

Test procedure:

A plastic paddle with four chambers or shallow cups used to perform the test. About 3 ml of milk directly striped into the labeled cups, LF, LH, RF and RH, from the respective four quarters. To ensure equal quantity of milk in each cup, the paddle should be tilted slightly at an angle of 45° to allow overflow of excess of the milk samples, if any in any cup. Then approximately equal quantity of the test reagent (CMT reagent) adds to each cup. The mixture of the milk and reagent is shaken gently in a rotating manner of the paddle in the horizontal plane.

Immediately after mixing or reaction must be scored within 15 seconds of mixing because weak reactions will disappear after that time. Any reactions of trace (T) or higher indicates that the quarter has sub clinical mastitis. The reaction is graded by intensity of gel formation as detailed below:

CMT score	Description	Interpretation
N (Negative)	No change	Healthy quarter
T (Trace)	Slime formed which disappeared with continuous movement of paddle	Sub clinical mastitis
1 (Weak)	Distinct slime, but no gel formation.	Sub clinical mastitis
2 (Distinct positive)	Viscous with gel formation, which adhered to the margin.	Serious mastitis infection
3 (Strong positive)	The gel formation with convex projection, the gel did not dislodge after swirling movement of the paddle	Serious mastitis infection



The upper most cup with the tenacious milk would be scored a 3 on the CMT

3. Strip cup test

This test is useful in field conditions for physical examination of milk. This test can be carried out by laymen at farm itself. In this test, enamel plate divided in four strip cups is used (Sharma *et al.*, 2009). The bottom of the plate is black colored so that it gives a good contrast to easily observe the milk flakes. The milk flakes can be seen by tilting the cups at an angle. This test is very useful in primary screening of animals for mastitis.



Flakes in the milk

4. Sodium lauryl sulphate test (SLS test)

This test is similar to CMT in principle as well as procedure. The difference is that in this test 3% sodium lauryl sulphate is used with instead of CMT reagent. This solution (test reagent) is prepared by adding 3 gm of sodium lauryl sulphate powder to 100 ml of distilled water. The suspension was heated to 50°C so as to make a clear solution. The pH of the solution was adjusted to 8.0 by using HCL or NaOH as per the need.

5. White side test (WST)

This test is also simple and easy to perform. The principal of this test is also similar to CMT. Take one drop of 4% NaOH to 5 drops of milk to be tested on a clean glass slide and mixed vigorously with a glass rod for 20 seconds. The development of a "viscid mass" was regarded as positive. The results are graded on the basis of precipitation of milk:

Grading	Description
Mixture remains opaque and free of particles	(-) Negative
Fine dispersed particles on close inspection	(+/-) Trace
A definite thickening and mixture separates into a milky whey and white particles	(+) Distinct positive
Mixture thickens immediately and follows glass rod.	(+ +) Strong positive

6. Surf field mastitis test

This test was developed by Muhammad *et al.* (1995). This test is easy, cheap and enough sensitive to detect all cases of sub clinical mastitis. The advantage of this test is that the readily available house hold surf (detergent) is used as reagent. The principle of this test is the reaction of somatic cells DNA with detergent (surf) and leads to the formation of gel of varying degree depending upon the number of somatic cells in the milk.

Procedure –

1. 3% Surf solution- Dissolve 3 g of surf in 100 ml of clean tape water or 6 teaspoonfuls of house hold detergent, surf in 500 ml of clean tape water. The test solution is stable for 6 months at room temperature.
2. A plastic paddle with four receptacles for the respective quarters of an animal can be fabricated from locally available plastic or bakelite. When this paddle is not at hand, suitable container such as tea cups or glass may be substituted.
3. Take equal quantity of 3% reagent and milk in the paddle or container. The mixture is swirled for about 1 minute and then examined visually for the presence of small floccules and gel. If floccules or gel is formed it indicates the positive for intramammary infection. In absence of any floccules or gel, sample is negative.

TREATMENT

Effective and economical mastitis control programs rely on prevention rather than treatment. Nonetheless, therapeutic intervention is an important part of a control program for bovine mastitis. Therapy of infectious disease should either

FARM MANAGEMENT & DISEASES

assist host defenses in eliminating invading pathogens and/or reduce the pathophysiologic consequences of infection. Logically, research emphasis and clinical application of antibacterials for therapy of mastitis has focused on the elimination of infectious agents. The potential goal of therapy is to attain clinical cures, with or without bacteriologic cures. 'Cure' means disappearance of clinical signs, elimination of infectious cause plus return to normal function and productivity. This may be desirable to promote the marketing of an affected cow's milk or to ameliorate the effects of a severe or life-threatening intramammary infection. Most clinical mastitis treatment protocols are based on treating the mastitic quarter to clear the infection and return the milk and quarter to normal. There are very few dairy procedures culturing clinical cases; therefore, most treatments are based on clinical signs. Performing cultures prior to treatment and basing the treatment protocol on the diagnostic results is not generally done because it is difficult to receive the results from the diagnostic laboratory quickly enough to allow their use prior to treatment.

Selecting clinical mastitis treatment is generally based on clinical signs, number of episodes and the likelihood of response. Clinical mastitis can be scored by severity with a score of 1 given when only the milk is abnormal, a score of 2 given when milk and quarter appearance are abnormal, and a severe score of 3 given when the animal is sick (Sears and McCarthy, 2003). Clinical mastitis treatment should include supportive therapy, milk-out, and observation until culture results are available the following day. When clinical mastitis cases are mild (a score of 1 or 2), treatment with oxytocin, with the quarter milked out, is generally sufficient until culture results are available (American Association of Bovine Practitioners protocol) (Morales *et al.*, 2001). Only more severe cases (with a score of 2 or 3) need immediate attention if the cow exhibits pain and fever. These cows should be given an anti-inflammatory (i.e., Meloxicam) to reduce fever, given fluids if necessary, and be rechecked in 12 hours. Further therapy decision can be made when the culture results are available the next day.

Antibiotic therapy

For antimicrobial therapy with either a parenteral or intramammary preparation, or the concurrent use of both types of preparation, to be effective an antimicrobial concentration exceeding the MIC for the causative pathogenic micro-organism must be maintained at the site of infection for an adequate duration. Antimicrobial therapy is pivotal for its containment and recovery. Despite the wide spread use of these drugs, antimicrobial treatment of mastitis has been less effective than desirable.

Parenteral administration-

Severe mastitis is usually treated systemically, although intramammary therapy will often be used adjunctively. The goal of antibacterial therapy is to attain effective concentrations of the drug at the site of infection. For bovine mastitis, there are three potential therapeutic targets, or pharmacologic compartments. The **first** (and most commonly targeted compartment) consists of the milk and the epithelial lining of the ducts and alveoli of the mammary gland. Pathogens (*Streptococcus agalactiae*, *Streptococcus dysgalactia*, *coagulase-negative staphylococci*) that typically reside in this compartment are generally noninvasive and are not believed to cause abscess formation in the parenchyma.

The **second** compartment consist the deep tissue of the mammary gland. Systemic administration is typically indicated for pathogens such as *Staphylococcus aureus* or *Streptococcus uberis* that are invasive or create abscesses. Cefquinome, a fourth-generation cephalosporin that has good tissue distribution and low MIC for gram-negative bacteria, was determined to be beneficial in reducing deleterious clinical outcomes of experimentally induced *Escherichia coli* mastitis. Recent evidence has suggested that the primary target for the treatment of severe coliform mastitis should be the **third** compartment of mastitis therapy: the cow. Bacteremia can occur as a consequence of coliform mastitis, and studies of naturally occurring cases have reported beneficial clinical outcomes for cows treated with oxytetracycline and ceftiofur (Cebra *et al.*, 1996).

An ideal antimicrobial for systemic therapy of mastitis should have the following properties (Ziv, 1980)-

- (1) Low MIC against the majority of mastitis pathogens
- (2) High bioavailability following intramuscular injection.
- (3) Lipid-soluble and predominately non-ionized in the blood.
- (4) Be sufficiently lipid soluble.
- (5) Have a low degree of binding to plasma proteins.
- (6) A long apparent half-life to ensure that concentrations above (preferably several-fold) the MIC are maintained at the site of infection throughout the dosage interval (12 or 24 hours).
- (7) Short withdrawal periods (milk withholding and slaughter).
- (8) Minimal adverse effects in cows.

Systemically administered sulfonamides, penicillins, aminoglycosides, and early-generation cephalosporins do not readily penetrate the mammary gland. Macrolides (erythromycin, tilmicosin), trimethoprim, tetracyclines, and fluoroquinolones distribute well to the mammary gland, although only the fluoroquinolones have broad spectrum of activity against many gram-negative pathogens. Fluoroquinolones, however, are undergoing intense scrutiny for use in food-producing animals because of bacterial-resistance concerns for humans. The sensitivity of common mastitis pathogens against different groups of antimicrobials is given in table 2.

Table 2. Group-wise comparative sensitivity of mastitis isolates

Group of antimicrobial agents	Name of antimicrobial agents	Staphylococcus spp.	Streptococcus spp.	Gram positive Bacilli	Gram negative Bacilli	Mixed isolates
Floroquinolones	Enrofloxacin	92.85	74.44	76.67	76.47	91.30
	Pefloxacin	70.58	83.33	78.42	91.66	87.24

FARM MANAGEMENT & DISEASES

Aminoglycosides	Kanamycin	86.66	86.11	88.90	86.67	85.72
	Neomycin	85.37	75.00	90.48	89.74	90.16
	Streptomycin	80.00	66.66	77.27	88.23	81.25
	Gentamicin	72.22	74.35	85.71	80.00	81.42
Microlides	Erythromycin	75.00	77.14	50.00	38.46	61.29
Cephalosporins	Cefotaxime	89.13	67.64	30.77	67.64	58.20
	Cephalaxin	68.42	67.74	42.86	66.66	56.75

All figures are in percentage

Source: Jha *et al.* (2004)

Drugs are transferred from the general circulation to the mammary gland by passive diffusion (Baggot, 1977; Ziv, 1980). Both non-polar lipid-soluble compounds and polar substances with sufficient lipid solubility passively diffuse through the predominately lipoidal barrier (Baggot, 1977). At a moderate level of milk production the ratio of the volume of blood circulating through the mammary gland to the volume of milk produced has been estimated to be 670:1; this provides ample opportunity for drugs to passively diffuse from the general circulation into milk. The rate of transfer is directly proportional to the concentration gradient across the blood-milk barrier and the lipid solubility of the drug. The milk-to-plasma equilibrium concentration ratio of total (non-ionized plus ionized) drug is determined by (i) the degree of ionization of the drug, which is pKa/pH-dependent, in blood and milk, (ii) the charge on the ionized moiety and (iii) the extent of binding of plasma proteins and milk macromolecules. It has been shown that only the lipid-soluble, non-ionized moiety of a weak organic acid or base that is free (not bound to proteins) in the plasma can diffuse through the cellular barriers and more readily reach the mammary gland. Majority of the antibiotics and the antibacterials are weak organic electrolytes, either acids (Amoxycillin, Ampicillin, Cloxacillin, Penicillin G, Novobiocin, Sulfanilamide) or bases (Tylosin, Lincomycin, Spiramycin, Erythromycin, Trimethoprim, Aminoglycosides, Polymyxin B) tetracyclines are amphoteric compounds and chloramphenicol is neutral (table 3). Fluoroquinolones (enrofloxacin, pefloxacin) are lipophilic and possess low degree of ionization. Accordingly in normal lactating cows (milk pH 6.5-6.8) weak acids attain milk and plasma concentration ratio of less than or equal to 1 because of their ionization at normal blood pH whereas organic bases excluding aminoglycosides (streptomycin, gentamicin, kanamycin, neomycin) and spectinomycin attain milk to plasma ratio of greater than 1. (Randhawa *et al.*, 2000). Therefore, lipophilic bases are preferred antibiotics because of these attaining higher concentration in milk. Although this favoured distribution decreases with increasing pH of mastitic milk, particularly for macrolides, but antibacterial activity of macrolides and aminoglycosides is not interfered.

In the presence of mastitis, the pH of milk increases to within the range 6.9 to 7.2. As a consequence the iontrapping effect on lipophilic organic bases is reduced while the concentrations attained by weak organic acids are somewhat increased. The inflammatory reaction in udder tissues enhances the passage of penicillins into milk. The increased pH of milk does not affect the concentrations attained by amphoteric drugs (fluoroquinolones, tetracyclines, rifampicin), but antimicrobial activity of these drugs is lower in milk than in extracellular fluid or *in vitro* determination would predict.

Table 3. Distribution of antibiotics throughout the udder after parenteral administration (Ziv, 1980).

Good	Moderate	Poor
Erythromycin	Amoxycillin	Dihydro-Streptomycin
Trimethoprim	Ampicillin	Neomycin
Spiramycin	Penicillin G	Gentamicin
Flouroquinolones	Suphonamides	Polymyxins
	Cephalosporins	
	Tetracyclines	
	Rifampicin	

Intramammary administration -

Intramammary route is accepted as the route of choice in the treatment of subclinical, chronic or mild clinical mastitis and as prevention during dry cow therapy. Intramammary administration permits delivery of the antibiotic directly to the mammary gland. However, Intramammary preparations are used, often in conjunction with parenteral preparations (depending on severity of infection), in peracute and acute mastitis. Intramammary antibiotics are distributed unevenly in an inflamed gland due to inflammation, swelling, and fibrosis that can block milk ducts, thereby preventing antibiotic diffusion throughout the gland (Owens and Nickerson, 1989; Owens and Nickerson, 1990). In addition, the intracellular location of some bacteria such as *Staphylococcus aureus* means that estimates of *in vivo* milk phase concentrations may not accurately reflect intracellular concentrations (Owens and Nickerson, 1989). Because antibiotics have variable penetration to the site of infection in mastitis (particularly in chronic infections), it is likely that MIC values determined *in vitro* do not accurately reflect the *in vivo* response to therapy. The degree of distribution of commonly used antibiotics after intramammary administration is given in table 4. Use of oxytocin for complete emptying the udder is highly desirable before intramammary infusions. Following intramammary infusion antimicrobial with high lipid solubility (macrolides, trimethoprim, fluoroquinolones) are readily taken up by and distributed widely in mammary tissue.

Table 4. Distribution of antibiotics throughout the udder after intramammary administration (Ziv, 1980).

Good	Moderate	Poor

FARM MANAGEMENT & DISEASES

Erythromycin	Penicillin G	Bacitracin
Trimethoprim	Cloxacillin	Dihydro-Streptomycin
Spiramycin	Cephapirin	Neomycin
Quinolones	Cephacetril	Gentamicin
Amoxicillin	Tetracyclines	Polymyxins
Ampicillin	Other sulphonamides	
Suphonamides		
Tylosin		
Lincomycin		
Amoxicillin-clavulanic acid		

Limitations of Intramammary administration

For the treatment of clinical mastitis only intramammary administration of antimicrobials is not sufficient to cure the animal completely because this route have some limitations like-

- Most of the products labeled for intramammary administration have been designed for use against gram-positive cocci and, thus, have little or no activity against gram-negative pathogens.
- Intramammary infusion with preparations that are not prepared under sterile conditions, however, can result in suprainfections caused by secondary invaders. Often, the secondary IMI caused by such pathogens as *Pseu. aeruginosa*, *Candida* sp, and *Nocardia* sp result in worse clinical implications than if no intramammary infusion was administered at all.
- Additional limitations of intramammary administration are encountered with more chronic IMI. Fibrin casts and microabscess formation interfere with distribution of infused drugs to the site of infection in the terminal alveoli.
- Furthermore, the typical 24-hour to 36-hour duration of therapy for intramammary infusions limits the time period of effective concentration in the gland necessary to eliminate more chronic or invasive IMI.

It is for these reasons that systemic administration of antibacterials has received attention as an adjunct or alternative therapy to intramammary therapy.

Intramammary preparations are formulated in forms as quick release of the antimicrobial agent or slow release of the antimicrobial over an extended period. The former are used mainly in lactating cows while the latter are used at the end of lactation (after the last milking) and in dry cows. Intramammary preparations for use in lactating cows should contain a readily available form, usually water soluble salt, of an antimicrobial agent with a low degree of binding to milk and mammary tissue proteins. The vehicle used and viscosity of the formulation should allow rapid release of the drug and ensure that effective concentrations are maintained throughout the recommended dosage interval (O'Rourke and Baggot, 2004).

Slow-release intramammary preparations may contain an antimicrobial agent with a high degree of binding to the secretions and mammary tissue proteins. Either a poorly soluble salt of an antimicrobial agent may be used or the formulation of the preparation be such that the rate of antimicrobial release is relatively constant, approaching zero order. The formulation of slow-release preparations determines the antimicrobial concentration-time profile in the mammary gland to a greater extent than quick-release preparations. Since only a single dose of a slow-release preparation is infused, the antimicrobial content is generally higher than in quick-release preparations. The antimicrobial must remain active (be stable) throughout the extended duration in the udder and the preparation must not cause irritation.

Supportive therapy

Anti-inflammatory : Anti-inflammatory drugs are widely used to treat acute clinical cases of mastitis. Most of the drugs currently in use inhibit the synthesis of eicosanoids, which include leukotrienes, prostaglandins, and thromboxanes. Arachidonic acid, which is located in the cell membrane of mammalian hosts, is the principal precursor for eicosanoids. Bacterial endotoxins activate phospholipase A2, which causes cell membranes to release arachidonic acid and make it available for the synthesis of eicosanoids. Eicosanoids contribute to localized and systemic inflammation, which may culminate to multiple organ-system failure.

In India, previously Diclofenac sodium was commonly used as anti-inflammatory drug but now it has been banned due to residual effect in treated carcass. Presently meloxicam is commonly used as anti-inflammatory in the treatment of mastitis at the dose rate of 0.5 mg/kg b. wt. intramuscularly.

Steroids : Glucocorticoid drugs inhibit the production of inflammatory molecules and the adhesion molecules that facilitate transport of inflammatory cells from the bloodstream to the site of inflammation. Glucocorticoids also help maintain microcirculation and cell membrane integrity, interfere with dissolution and disruption of connective tissue, decrease formation of histamine by injured cells, and antagonize toxins and kinins. These

actions of glucocorticoid drugs could be expected to be of benefit to the mastitic cow, particularly one with mastitis caused by endotoxin-producing coliform bacteria (O'Rourke and Baggot, 2004). Glucocorticoids need to be administered early in the course of disease for maximum efficacy. Intramuscular administration of Dexamethasone @ 30 mg (as total dose for adult cow) is sufficient.

Oxytocin : Milk is the rich medium for the growth of microorganisms, hence it should be removed from the affected quarters at every 3-4 hours. Oxytocin used to facilitate complete milk evacuation and to remove toxic material and debris. Intramuscular administration of oxytocin @ 40-50 IU per animal per day.

FARM MANAGEMENT & DISEASES

Others : Srinivasan (1998) found promising result with the intramuscular use of L-ascorbic acid (Vitamin C) @ 25mg/kg b. wt. in the management of subclinical mastitis. Sharma *et al.* (2007) have been reported that dietary supplementation of vitamin E (500 IU/animal/day) and Selenium (6 mg/animal/day) effective for the treatment and control of subclinical mastitis in lactating dairy cows.

Fluid therapy : Isotonic fluids containing glucose and antihistaminic are to be used in peracute and acute cases with extensive tissue damage and toxemia. Intravenous infusion of hypertonic saline (7.5% NaCl) @ 2ml/45kg b. wt. is useful in the treatment of acute mastitis. Intramammary administration of 0.5-1.0 liter of hypertonic saline in affected quarter once a day after milking for 2-3 days is clear the 50-60% of the clinical symptoms. Application of cold (ice bags) reduces the absorption of toxins in peracute and acute cases of mastitis.

PREVENTION AND CONTROL

The fundamental principle of mastitis control is that the disease is controlled by either decreasing the exposure of the teat ends to potential pathogens or by increasing resistance of dairy cows to infection. Controlling mastitis is not a matter of doing just one thing. Instead, it involves following a number of steps better referred to as a control programme. To be acceptable, such a programme must be economical, practical, effective under most management conditions, and reduce new infections. The programme should also shorten the duration of pre-existing infections, reduce the incidence of clinical mastitis, and be subject to easy modification as improved methods are developed through research. Fortunately, working toward the reduction of clinical mastitis is not in conflict with the long range objectives of a control program because most clinical cases are preceded by sub clinical cases. Thus, a reduction in the more obvious clinical mastitis is evidence that the unnoticed sub clinical infections are also being reduced.

Controlling mastitis is not a matter of doing just one thing. Instead, it involves following a number of steps better referred to as a control programme. To be acceptable, such a programme must be economical, practical, effective under most management conditions, and reduce new infections. The programme should also shorten the duration of pre-existing infections, reduce the incidence of clinical mastitis, and be subject to easy modification as improved methods are developed through research. Fortunately, working toward the reduction of clinical mastitis is not in conflict with the long range objectives of a control program because most clinical cases are preceded by sub clinical cases. Thus, a reduction in the more obvious clinical mastitis is evidence that the unnoticed sub clinical infections are also being reduced.

A key factor in preventing mastitis is to reduce the number of bacteria present around the teat end. This is particularly important in the period from about two weeks before calving to two weeks after. The following points should be practiced to control the mastitis-

1. Preparation of animal

Clipping the hair on the udders, the flanks and inside the hindlegs. Before milking animal should be clean.

2. Milk cows with clean, dry teats and teat ends-

Proper milking procedures are important for the prevention of mastitis and for insuring complete milk removal from the udder. Teats and teat ends should be washed with water and dried completely before the milk is taken by hand or machine. Emphasis should be placed on the teat ends. An additional positive step may be predipping with a sanitizing solution similar to those used for post-milking teat dipping. Predipping with teat dip has become popular. The procedure for predipping involves washing of teats with water and a sanitizer. The teats are then dried with an individual paper towel and dipped or sprayed with the sanitizer. A 30-second contact with sanitizer is needed to kill organisms. Then the sanitizer is wiped off with a paper towel. Predipping may be beneficial in reducing mastitis, but the actual dipping, dip contact time, and wiping with a towel increase the total milking time. If the dip is not wiped off, excessive chemical residues in milk may occur. If contact time is not sufficient, then it's a very expensive premilking regime. There are many effective teat dips, including iodine at 0.1%, 0.5%, and 1.0%. Also, although it is not labeled for teat dipping, hypochlorite at 4.0% with a sodium hydroxide content less than 0.05% was effective in field trials. There are many more teat dips on the market that are effective in preventing new infections. Effective coverage of the teats is more important than the type of dip being used.

3. Prevent transfer of pathogens from cow to cow during milking

This has been universally successful to control most contagious mastitis pathogens by preventing spread from one infected cow to non-infected cows during the milking process. Single use towels (paper, cloth, wipes) should be used in preparation of the udder and teats. Gloves for milkers are helpful and should permit constant washing with irritation to hands. Separate milking units for infected cows, established milking orders to protect the non-infected cows, hospital pens with separate milking equipment are all appropriate methods. Hospital cows should not be milked with the fresh cows. Post-milking teat dipping is very important. There is only one way to effectively stop the spread of mastitis in the dairy herd, and that is by applying teat dip to every quarter of every cow after every milking. Teat dips are used to remove milk residue left on the teat and kill organisms on the teat at the time of dipping. They also leave a residual film of sanitizer between milkings. Post-milking teat dipping is effective in eliminating environmental organisms *E. coli* and *Strep. uberis* on the teats after milking.

4. Prevent injury to the teats and udder during milking

Any injuries to the teats or teat ends and udder will eventually end up with a new case of mastitis. Important steps are proper milking techniques (attachment, alignment and removal of machines), proper milking machine design/function, routine and timely changing of inflations, and continuous maintenance of the milking equipment (cleaning pulsators, etc.). Periodic assessment of teat end condition may be a useful indicator. Environmental sources of injuries should also be controlled (bedding, housing, free stall design and maintenance, frostbite).

5. Housing

Providing adequate space, ventilation, bedding, and lighting to ensure cleanliness and comfort at all times. Over crowding should be avoided.

6. Early detection of new infections (clinical and subclinical) and treatment

Prompt detection of mastitis will preclude severe mastitis outbreaks. Milking may begin with a check of all quarters for mastitis. Any cows that show clinical mastitis should be examined and appropriate action taken. If fore milking is not done, visual checking for inflamed quarters is done by milkers and herd health people. Early detection may be by prestripping prior to milking, observation of the udder and teats, California mastitis test, various forms of electronic somatic cell counting or electrical conductivity. Milkers should be trained to use these techniques and management feedback is important.

Just after confirmation of mastitis treatment with appropriate therapy should be started as early as possible.

7. Milking

Cows should be milk completely to prevent the occurrence of new cases. Standard milking techniques should be used. Follow proper milking procedures, milk clean, dry udders, apply milking units properly, and make adjustments to prevent the admittance of air into the teat cup liners and prevent liner slip.

8. Provide adequate nutrition to preclude increased susceptibility to mastitis

The mammary gland can resist most infections if it is adequately supplied with the essential nutrients it needs to maintain resistance to new infections. Those microminerals which are important are selenium, copper, zinc, vitamin A and vitamin E. When these are not supplied in adequate quantities, the rate of new infections may increase.

Suggested levels of supplementation (amount/cow/day):

Selenium	6 mg (Lactating cows)
	3 mg (Dry cows)
Copper	200-250 mg
Zinc	900-1200 mg
Vitamin A	100,000 – 150,000 IU
Vitamin E	500 IU (Lactating cows)
	1000 IU (Dry cows)

9. Fly control

Some flies spread the infection from infected cow to healthy one, particularly summer mastitis and other pathogen including *Staphylococcus aureus* from one source to the teat ends of heifers or cows. They can also cause sites for infections by biting the teat ends. Basic fly control involves prevention of breeding sites through routine removal of manure and decaying feeds. Insecticide ear tags and sprays may also help.

10. Vaccination

Vaccination is an important aspect in disease prevention but in mastitis vaccination is not much successful due to its multifactorial complex etiology. However, vaccine against major mastitis pathogens like *Staphylococcus aureus*, *Streptococcus uberis*, *E. coli* have been developed, but they are costly.

11. Dry cow therapy

Dry cow treatment is administered after the last milking of the cow before the dry period. Care must be taken to scrub the teat end with cotton and alcohol before infusion and to use teat dip after infusion. There are many antibiotics available for dry cow therapy. High levels of penicillin and dihydrostreptomycin, the cloxacillins and other products specifically for dry treatment are effective. The idea of dry period therapy has been accepted because antibiotics can be put into a slow release base that allows them to stay in the udder longer. They are not constantly being milked out of the udder as is the case with lactation therapy. Antibiotics can be administered in higher quantities because there is no concern for milk levels and antibiotic residues. Management of dry cows also is very important in mastitis control. If dry cows are exposed to muddy or dirty conditions, risks of mastitis will increase. This is especially true at the time of calving; cows are under much stress during this period and if an udder is exposed to wet dirty conditions, mastitis will increase. If you believe that your dry cow therapy program is ineffective, it may be because of poor treatment procedures and/or improper management of the cows during the dry period and at calving.

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