

Menace of mastitis, risk factors and selective therapeutic strategies

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Abstract

Mastitis is an infectious disease of udder and teat caused mainly by bacteria in milk yielding animals. It is an acute disease but sometimes it may extend to chronic condition, Clinically the disease characterized by swollen udder, teat and occasional septicemic condition with physical and chemical changes of milk. Several bacterial species both Gram negative and positive cause the condition. The common bacterial infections are *Staphylococcus* sp, *Streptococcus*, sp, *E.coli*, *Mycoplasma* sp., *Klebsiella* sp, *Corynaebacterium* sp and others. For effective treatment of mastitis both chronic and acute cases need selection sensitive antibacterial drugs primarily 4th generation onwards of penicillins and supporting treatment anti-inflammatory drugs, steroids, isotonic glucose (5%), hypertonic saline (7.5%) with immediate adequate drinking water. Modern drugs of nano molecules and sound therapy against mastitis are on advance trials. Mineral and vitamin mixture supplements can enhance recovery of the condition. It is better not to occur the disease than treatment, therefore, need cleanliness of animals and fomites along with regular checking of the milk composition for early detection of mastitis.

Introduction

Mastitis is the inflammatory pathological changes of mammary glands in dairy animals particularly in bovine, caprine, ovine and other milk yielding animals. The condition mostly occurs in and around post parturition period when udder and mammary gland activities is more. It is a mostly acute high devastating infectious disease and has potential transmission of deadly infection such as tuberculosis, brucellosis, leptospirosis to human and animal consumers of milk. The high yielding animals like cattle and buffalo females are at very risk as these animals produce much milk and provide back bone of dairy industry, even small farmers who rear small herd for livelihood may fall into this highly mastitis menace that ceased the milk production and spoil the animals also. Indian dairy depends on some specific breeds of high yielding cattle such as red Sindhi, Sahiwal, Deoni, Jersey, Holstein Friesian etc. along with local breeds of cattle and buffaloes where the epizootiology of mastitis is highly prevalent (Kurjogi, *et al.*, 2014).

It causes several fallouts of the disease directly and indirectly with loss of milk production, low milk, milk quality and muscle production, deterioration of physical health status, extra cost for therapeutics and nutrition therapy, case fatalities, reproductive loss for subsequent pregnancies and affect the calf's health status (Gomes and Henriques, 2016, Azooz, *et al.*, 2020). The highly infectious disease may spread to the new hosts and for other pathological condition due antimicrobial resistance against many antibiotics use haphazardly (Ameen *et al.*, 2019). This article is concerned with causes of mastitis, timely diagnosis and needful therapeutic intervention at proper time.

Epizootiology

Mastitis is worldwide infectious disease; the prevalence rate may be as high as 50% and low at 10% depending on herd size, sanitation and health coverage. The non lactating heifer may be affected 30-40%. The case fatality rate varies depending upon the virulence of bacteria and health status.

The infections may be with contagious established pathogens and environmental pathogens which lasts not much long in the host such as *Streptococcus dysagalactiae*, *S.uberis*, Staphylococcus chromogens etc. The infections may be within udder and environmental contamination. Seasonal variation of the incidence may vary post rainy and pre-winter seasons with confined herd found more prevalence (OldeRiekerink, *et al.*, 2007). Older animals are more susceptible than heifer with mastitis. Several other risk factors have also contributions in the prevalence of mastitis.

Risk factors

Various risk factors are involved in the incidence of mastitis in animals. **Animal** risk factors such as age of animal, numbers pregnancy, age of animal. **Stage of lactation**-new infections occurs in early part of lactation and may occur during last trimester of gestation. Persistence prevalence of infection in a herd has more occurrence of new infection. Breed variation- Holstein-Friesian, Jersey are more susceptible than native breeds. High yielding animal with wide teat canal tends to more infection. Nutritional supplement with immunostimulant nutrients such as vitamin E, A and Selenium can prevent infection to some extent (Kitila *et al.*, 2021). Previous infection has some relation with subsequent infections. Housing and quality management of animal affects the incidence rate. Effects on animal health and welfare. Keeping the herd clean and comfortable can reduce the incidence and severity of mastitis. Stock density per unit space, wet contaminated bedding, and improper ventilation can invite mastitis. Milking practice-such as machine milking, wet teat and soiled udder, after milking sanitization of teat and udder are some of the importance issues for mastitis

Species of animals affected -all most all domestic mammalians are affected. For production purpose cattle, sheep, goat, buffalo are cross bred for milk and meat production. These animals are more susceptible than swine, bitch, mare, rabbits and others. Buffalo mostly shows subclinical mastitis.



Types of mastitis

Bacteria that dwell in the skin, teat and udder can be transferred one to other host and can cause pathology of udder and teat tissues. Accordingly, the severity and duration of the pathological conditions the mastitis can be classified as contagious mastitis, environmental mastitis and summer mastitis. Contagious mastitis is categorized as clinical mastitis, sub-clinical mastitis and chronic mastitis.

Clinical mastitis

Characterized by swelling swollen, warm and reddened udder, painful condition, milk clot, discoloration of milk, septicemic condition, fever, loss of appetite and depression. Proper milk not letting out, consistency of milk is deviated; there may be blood-stained milk. This condition is treatable with good response. Clinical mastitis may be of three types, per-acute, acute and sub-acute

Sub-clinical Mastitis

this type of mastitis characterized by presence of pathogenic bacteria in the mammary tissue but with characterized clinical signs of inflammation, swelling of udder swelling and systemic reaction. Here the somatic cell counts, leucocytes and epithelioid cells may be increased but remains more than 10^5 /ml milk. Apparently, milk composition except cellular components remains unchanged but yield may be reduced.

Chronic mastitis

Here the infections persistently remain inside the mammary tissue show little healing tendencies of the tissue reaction (fibrosis) for months even many lactations. A particular quarter of udder is affected. Sometimes from this condition an acute mastitis may flare up. This type of mastitis provides little response to treatment.

Again, there are other types of mastitis that affect the transmission of organism occasionally, they are **Environmental** mastitis and **Summer mastitis**. Environmental mastitis where the microbes not dwell in the host system but remains plenty in the environment and contaminates the udder when the host's teats come in contact with environment. The summer mastitis is an acute severe painful swollen udder with high tissue damage permanently. This type mastitis infection is transmitted by a vector fly called *Hydrotaea irritants*. The summer mastitis occurs mostly in sandy soil areas where the head fly (sheep) breeding den and the bacteria vectored are *Corynaebacterium pyogenes*, *Streptococcus dysagalactae*, *Peptostreptococcus indolicus*. The summer mastitis characterized by hot, hard and swollen quarters and also some cases of systemic involvement in dry cows preferably.

Causes of mastitis

A long list of bacterial pathogens causing mastitis. Pathogenic bacteria those cause mastitis in animals are divided into three categories (i) Major pathogens (ii) Minor pathogens (iii) uncommon pathogens



(A) Major pathogens

Gram positive bacteria-*Streptococcus agalactae, Streptococcus equinus Streptococcus dysagalactae, Staphylococcus aureas, S.suis, Streptococcus uberis, Streptococcus equines, Enterococcus faecalis , Enterobacter faecium, Eterobactor aerogenes, Enterobacter fecalis, Endurance., E.cloaca,Arcanobacterium pyogenes, Lactococcus lacti.*

Gram negative bacteria -*Escherichia coli, Klebsiella pneumoniae, K.oxytoca , Serratia spp. Mycoplasma bovis and other mycoplasmas and Pseudomonas spp, Proteus sp, Yersinia spp. , Pseudomonus pyogenes etc*

Minor Pathogens

Gram positive bacteria- *Staphylococcus chromogenes, S. haemolyticus, S. epidermidis, S. simulans, S. sciuri*

Gram Negative bacteria-*Corynebacterium bovis, Pasturella multocida, Campylobacter jejuni, Fusebacterium necrophorum, Mycobacterium tuberculosis, M. fortuitum, M lacticola, Clostridium perfringens,*

Yeast- *Candida rugosa, Candida krusei, Candida glabrata C.zeylanoides, Cx. norvegica, C. viswanathii, C. guilliermondii, C.albicans, Nocardia farcinia,*

Fungi-*Aspergillus fumigates, A.nidulas;Cryptococcus neoformans,Actinomyces-Actnobacterium pyogenes*

Algae-*Prototheca trispora, P.zopfii,*

Non-infectious mastitis- Physical injury of udder and teat, granulomatous inflammation of udder

Pathogenesis

The defensive mechanism of teat is the teat sphincter at the teat opening. Bacterial transmission occurs through the teat opening and canal, the organisms multiply in the log phase and aggregate grapes like clusters most of the acute infection. The organisms also produce bacterial metabolites, toxins and enzymes causing inflammation of the tissues. Rapid multiplication of bacteria causes extension of new area of teat and udder tissue. The inflammatory reaction causes pain, swelling, fever, anorexia, shock, there may be block of milk passage and thick milk like secretion in acute cases while in sub-acute cases several local reactions, changes in milk consistency and micro clots and discoloration of milk. Chronic mastitis characterized by few clinical signs for long times even years and there are irreversible changes of mammary tissues. Infection in the early lactation may cause per acute mastitis with udder gangrene formation. During late lactation and dry period no new infection occurs with systemic reaction. The acute and per acute mastitis necrosis of involved tissues cause tissue oedema, congestion and toxemia with *Staphylococcus sp, E.coli, Clostridial spp* involvement. Both acute and per acute cases, proliferation of bacteria in collecting ducts and alveoli occur, as a result small ducts are clogged with fibrin that led to blockage of milk flow. The interstitial of the alveoli in infiltrated with lymphocytes, macrophages, plasma cells and fibroblasts. In mild pathogenic



bacterial infection, the reverse of tissue reaction and heals to normal tissue within few days to weeks. In chronic form, inflammatory reaction is very slow and restricted to epithelium of ducts that subsides within some days replacing connective tissue proliferation around ducts that also blocks to flowing areas. In chronic cases, cyclically shedding of bacteria from the affected quarters and some cases an abscess may develop (Gomez *et. al.*,2016, Vidlund *et al.*,2022).

Clinical symptoms

Clinical signs of the mastitis vary widely as the causes of mastitis are many pathogens. Some are common highly pathogenic, while others may be mildly pathogenic. Therefore, the clinical signs vary with individual bacterial species. Acute and per acute clinical signs exert by various highly pathogenic bacteria such as *Staphylococcus* sp, *Streptococcus* sp. The chronic form of mastitis may be due to fallout of an acute infection or slow multiplying bacteria.

Acute and per acute

clinical signs with severe swelling of udder, teat erected, milk is purulent with formation of clot with bloody stain. Due to severe reaction udder tissue shows fibrosis and functionless (Fig-1). This condition usually occurs postpartum with high yielding animals. Due to highly pathogenic bacterial invasion, a fatal systemic reaction occurs with high fever (40-42°C), tachycardia, anorexia, depression, ruminal atony, weakness, recumbency, udder affecting quarter swollen, firm and hot. Udder gangrene may eventually occur with cyanotic hue. The gangrenous tissue may ooze serum with blister formation. Thrombus formation in the blood vessels cause gangrene as result toxaemia develops and the animal may die. The milk of the affected animals may be watery, flakes, micro clots, cellular infiltration and pus in the milk (Fig-2). In very severe cases gangrene of udder and teat happens, here even unaffected quarters may be affected with edematous swelling and mammary vein.

Sub clinical mastitis- This form of mastitis characterized by inflammatory reaction in udder, the milk color also not much changed however the milk composition and cellular counts increased.

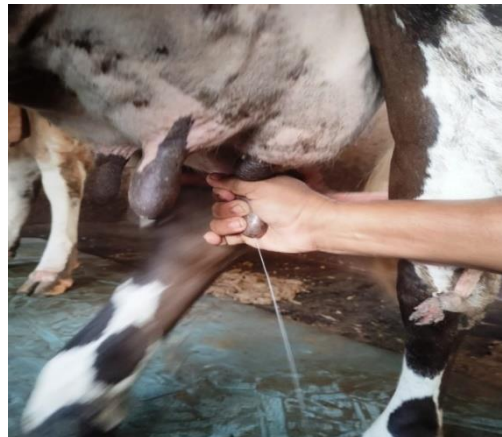


Fig-1: Swollen udder, erected teat, and lumpy quarter **Fig-2:** Milk flakes eliminated from teat

Chronic mastitis

In chronic mastitis the tissue and pathogenic reaction is slow and fallout of a previous infection. Clinically, udder may be the only parts of involvement without systemic reaction. The udder



and affected quarter may be atrophied with fibrous tissue. The fibrosis of tissue caused atrophy of the alveoli (basket / myoepithelial cells around the alveolus) and gland cistern as consequence the production of milk is ceased or reduced. Recovery from the fibrosis of udder is very scarce if not attempted very early of the disease course

Diagnosis

The disease can be diagnosed with clinical signs and palpation of the teat and udder. Swollen, hot, painful, firm udder, abnormal milk consistency, systemic reaction with fever, anorexia are common clinical signs. Changes in physical character and chemical characteristic of milk. Chronic cases of mastitis can be detected by intramammary inspection of udder and individual quarter with fibrotic tissue reaction and lumpy hard udder or its part. Some laboratory diagnostic methods are as follow for confirmative diagnosis.

Somatic cell count (SCC)

Somatic cell count of milk per ml by the microscopically. The number SCC may vary depending upon the number of cells present in the milk. Normally, the somatic cell count remains below 100000 cell/ml of milk. Increase in number indicates the pathology of udder.

Total Bacterial Count of Milk

The milk usually contains bacterial contamination. The number of bacterial counts ranges from 20,000 to 100,000 per ml of milk normally. The bacterial count of milk can be done by standard plate counting (media in plate inoculated with milk sample) on 24 hour incubation in dilution.

Culture method

For identification of organisms' milk samples may be collected from suspected individual animals and pool milk samples. Isolation and identification of microbial species can be done culturally, biochemically and serologically.

California Mastitis test (CMT)

It a quick diagnostic test to detect mastitis even the subclinical mastitis. It indicates the cell counts level of milk. The test reagents lyses the cells present in the milk and react with the DNA of the somatic cells forming a gel. The reaction graded from 0 to 3, 0 indicates unchanged of reaction while 3 indicates formation of solid gel in high number of SCC.

ELISA testing

ELISA for antibody in milk particularly the frequent infection *Staphylococcus aureus*, it is 87% accurate to indicate mastitis infection. The list is highly sensitive and specific to declare mastitis

Molecular method

PCR for identification of bacteria which may not be easily cultivable. The amplification of the bacterial biomarker targeting the 23S and 16S rRNA spacer region sequence (Phuektes *et al.*,2001)



Biosensor method

A biosensor is a device that can detect the biomarkers of a particular pathogen sensing the elements called bioreceptor (RNA, DNA, lectins, enzymes, whole cell, monoclonal antibody etc). The bio-receptor is critical biochemical properties component which is highly sensitive and specific to detect and avoid interference with other microorganism and molecules. The interaction between biomarker and bioceptor are converted into signal by the transducer. Signal detection and display allow to identify pathogens both qualitative and quantitatively (Hogeveen *et al.*,2021).

Use of BROLIS Herdline

It a continuous system of observation somatic cell count of milk regularly from individual milk. The changes of electric conductivity of milk with more laclose, potassium, sodium chloride and other component is estimated automatically by a robot. Even subclinical mastitis can be detected though this device before 1-2 weeks of onset of mastitis. The electrical conductivity of normal milk is 4.0-5.5 mS/cm. Damage of mastitic mammary cell releases more potassium, sodium chloride which increase the conductivity of electricity more than 6.0 mS/cm is an indication of mastitis. It helps early diagnosis and for control measure

Hymast diagnostic tes

This test is done to identify the bacterial type i e Gram negative or Gram-positive involvement. This test is needed to establish good therapeutic response. The kit is used to cultivate bacteria for differentiation of types. Gram positive bacteria can be treated with piramycin while Gram negative bacterial infection with tazobactam, carbapenems etc.

Therapeutic interventions

The microbial involvement in mastitis shows three types of clinical signs mild, moderate and severe ones. Mild and moderate mastitis can be treated with decisive therapy with antibiotics as per experience by the animal attendants. The severe form of mastitis treatment needs specialized attention for use of suitable sensitive antibiotics, anti-inflammatory drugs and proper clearance of abnormal milk and milk clot from the udder.

Use of antibiotic

Before starting antibiotic therapy, bacterial involvement should be visualized by the veterinarians. If possible, an antibiotic sensitivity test needs to be done to find out the suitable antibiotic application. Antibiotic can be given in two routes intramammary and intramuscular depending on the severity. Before applying intramammary infusion accumulated milk and debris from the udder should be removed for good response with antibiotic (Fig-2). Marketed preparation of mammary infusion antibiotic is available 5g gram for each quarter. This should be given for 3-5 days. In case of systemic involvement, a course antibiotic may be given parenterally. Antibiotic of beta-lactams (penicillins, piperacillin, tazobactam, clavulanic acid combination), tylosin (10mg/kg body wt) against mycoplasmal infection, cephalosporine (2-3mg/kg) and erythromycins (12.5mg/kg b wt)



are their higher generations are used. Repeated antibiotic therapy can reach antibiotic resistance where phytochemical can be used which are comparatively less resistant but action also not much prompt. Phytochemicals like cucumin, luteolin, thymoil can limitedly be used. Animal products like bee venom can induce COX-2protein expression to reduce pain, bee venom also have propolin has anti-inflammatory response.

Ant inflammatory

Several anti-inflammatory drugs need to be applied to relieve pain and swelling of udder also to reduce fever and toxaemia. Several non-steroidal anti-inflammatory drugs (NSAID) can be used, common such NSAID are Flunixin meglumine (2-3mg/kg b.wt), ketoprofen (3mg/kg b.wt), flurbiprofen(10 mg/kg), carprofen (1.4mg/kg b.wt) and meloxicam (5mg/kg b.wt). Effective but restricted amount of steroidal such as Prednisolone (10-20mg), dexamethasone (50mg),flumethasone (5mg) can be used in severe cases of mastitis through parenteral or intramammary infusion. The steroidal helps in removal of toxins and leucocytosis.

Prepartum antimicrobial therapy

Early administration antibiotic like cephalixin at 10–12-week prepartum for three days may restrict mastitis about 90%.

Use of antibiotic bound nanoparticles

Several nano particles are in the trail for different mastitis cases treatment. Ciprofloxacin-loaded Chitosan nanoparticles (0.15 µg/mL) for the treatment of bovine mastitis. The antibiotic nanoparticle delivery system helps to penetrate the antibiotics and nanoparticles into the bacterial cells. Both chitosan and antibiotics combinedly show synergistic effect of drugs destroying the bacterial population. Several other nanoparticle loaded with antibiotics or without antibiotics such as silver nanoparticle (AgNPs), Copper nanoparticles (CuNPs) are used against Mastitis caused by bacteria (Kalinska *et al*,2019)

Application of acoustic pulse technology

Acoustic pulse is one type of shock wave that can improve healing of mammary tissue particularly in chronic mastitis cases. The shock wave promotes recover of damage tissues by increasing secretion naturally anti-inflammatory cytokines (IL-1 β , IL-8, IL17 etc), neurovascularization, angiogenesis (more basculation). Total 400 pulse (2.5 minute/ treatment) shock wave is given (3Hz) for three days. Along with 30 ml of gentamicin or cephalixin are also used. The combined effect of antibiotic and shock wave improve fast healing of mastitis (Leitner *et al*.,2021)

Supportive therapy

Several supportive drugs can help in early recovery of mastitis along with primary treatment. Isotonic glucose (5%), hypertonic saline (7.5%) with immediate adequate drinking water. This improves renal function and more circulation. Sometime mineral and vitamin mixture (Mammidium)



also given that may help in changing of pH system, inhibition of growth of bacteria, tissue oxidative damage and regeneration of tissue.

Consequence of mastitis

Morbidity and mortality significantly impact milk production, weight gain, and the expenses associated with treating affected animals, constituting substantial losses due to any disease. However, mastitis gives rise to various other detrimental consequences. These include:

1. **Transmission of Infection:** The infection can spread to other susceptible hosts, affecting new animals.
2. **Delayed Fertility:** Animals may experience delayed fertility, accompanied by negative impacts on reproductive aspects of their lives.
3. **Increased Veterinary Costs:** Additional expenses arise from veterinary care and treatment.
4. **Necessity for Premature Culling:** The need for premature culling arises, leading to reduced overall production.

Control of Mastitis

Fly control measures to mitigate summer mastitis, typically involving synthetic pyrethroids, encompass various strategies like the use of impregnated fly tags, pour-on preparations, and sprays to effectively eliminate fly populations.

Continuous surveillance using tools like BROLIS Herdline, which monitors electric conductivity, can serve as a proactive approach for predicting mastitis incidents on both production farms and individual animals.

Maintaining the overall cleanliness and hygiene of the herd is imperative. This includes ensuring that bedding and the flooring of sheds or farmhouses remain clean and dry. Regular burning of the farm and fomites is also recommended to curb infection spread.

Consistent pre-milking and post-milking teat disinfection should be standard practice. Even during the dry period, teat dipping with sanitizers or disinfectants for the last two weeks of pregnancy is advised. Careful attention is necessary to prevent conditions like milk fever and downer cow syndrome, as these can also contribute to mastitis susceptibility. In areas where mastitis is endemic, considering vaccination with vaccines targeting highly prevalent bacterial strains may be a worthwhile practice.

Conclusion

Mastitis is a moderate to severe infection of udder with bacteria where an urgent treatment is needed with an effective and selective antibiotic, ant inflammatory and other supportive drugs. Very time effective early diagnosis and effective treatment can save the animal need selective and sensitive antibiotics and other drugs. Healthcare for pregnant and lactating animals is an obvious measure to control and restrict occurrence of mastitis.



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