An Overview on the Epidemiology and Diagnosis of Bovine Mastitis

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Introduction

Mastitis is the most common infectious disease encountered in intensively-farmed dairy cattle (Quinn et al., 2002). It is inflammation of the parenchyma of the mammary gland regardless of the cause. Mastitis is therefore characterized by a range of physical and chemical changes in the milk include discoloration, the presence of clot and present of large number of leukocyte (Radostits et al., 2007). In other words, bovine mastitis is a response to ascending infection of the gland by the way of the teat canal (Jubb et al., 1993). There is swelling, heat, pain and edema in the mammary gland in many clinical cases. However, a large proportion of mastitic gland is not readily detectable by manual palpation or by visual examination of the milk strip cup; these quarters represent subclinical infection (Radostits et al., 2007).

Etiology

A total of about 140 microbial species, sub species and serovars have been isolated from the bovine mammary gland. However, a relatively small number of them are responsible for most cases of mastitis (Quinn et al., 2002 and Radostits et al., 2007).

The bovine mammary gland is the principal reservoir of infectious agent which causes contagious mastitis, namely Staphylococcus aureus, Streptococcus agalactiae, Mycoplasma bovis and Corynebacterium bovis (Quinn et al., 2002). Staphylococcus aureus is one of the most causes of bovine subclinical mastitis (Wilson et al., 1997). Streptococcus agalactiae was a major economic loss in pre-antibiotic era. It remains a significant cause of chronic mastitis in many herds, even though it can readily eliminated (Jain, 1987). Mycoplasma bovis is the most important agent in primary mycoplasmal mastitis in cows (Jones et al., 1996).

Environmental mastitis is associated with three main groups of pathogen, the coliforms (particularly Escherichia coli and Klebsiella species), environmental streptococcus and Arcanobacterium pyogenes (Radostits et al., 2007).

Contagious mastitis is caused by bacteria which reside primarily in the mammary gland of cows whereas environmental mastitis is associated with micro-organisms which are present in the environment (Quinn et al., 2002).

Major pathogen

Contagious pathogens like S. agalactiae, S. aureus and M. bovis are major pathogens. Likewise environmental Streptococcus species (S. uberis and S. dysgalactiae) and the environmental coliforms such as E. coli and Klebsiella species are also major pathogens of mastitis (Radostits et al., 2007). Contagious diseases only remain endemic when the mean number of susceptible individuals infected by an infected individual is appreciably larger than one (Becker, 1989).

Minor pathogen

Several species of bacteria are often found colonizing the teat streak canal and mammary gland. They rarely cause clinical mastitis and are known as minor pathogen. They include the coagulase negative Staphylococcus species such as Staphylococcus hyicus and S. chromogenes which are commonly isolated from milk sample and teat canal. Staphylococcus simulans and S. epidermidis are part of the normal flora of the teat skin (Radostits et al., 2007).

Epidemiology

Mastitis is the major disease problem which appears to be worldwide distribution and affects all species of animals. It is a sporadic disease and attains major economic significant in terms of profound impact on the value and productivity in dairy animals (Radostits et al., 1994).

Source of infection

Streptococcus agalactiae and Staphylococcus aureus reside primarily in the udder of infected cows. The source of the infection is other infected cow and exposure to uninfected quarter is limited to the milking process. Streptococcus uberis, Streptococcus dysgalactiae, and coliforms are common inhabitant of the cow environment such as bedding. The exposure of uninfected quarter to the environmental pathogen can occur at any time during the life of the cow, including milking time, between milking, during the dry period (Radostits et al., 2007).
Method of transmission
The usual route of invasion is through the teat canal, the infection originating either an infected udder or the environment; in dairy cattle the infection originating from infected udder is transmitted to the teat skin of other cow by milking machine liners, milker hand, washing cloth and any other material that can act as inert carrier. The route of invasion may also be hematogenous as in tuberculosis and brucellosis (Jubb et al., 1993).

Risk factors
Important pathogenic risk factors include presence of number of organisms on teat skin and their virulence factors, presence of minor pathogens and blind treatment. The incidence of mastitis seems to be related to the number of organisms on the teat skin and teat end (McDonald, 1997). Streptococci and Staphylococci are in high numbers on teat skin; hence, they are the cause of most intra-mammary infections. The various reports indicated that the quarters that harbor minor pathogens are less susceptible to new infections by major pathogen than uninfected quarters, a phenomenon that is possibly related to the protective effect of the cell response triggered by the minor pathogens. Several studies have reported that infection by the minor pathogens Corynebacterium bovis and the coagulase negative Staphylococci can prevent subsequent infection with the major pathogens (Doane et al., 1987).

Udder infection may develop when the cow is lactating or dry. Infection rates are highest in early dry period although this infection may not persist or develop into clinical mastitis until the next lactation. The high incidence of mastitis around calving is largely a consequence of high new infection rate in dry period and a periparturient suppuruntation of host defenses. Increasing disease with age is probably not due to increased intra-mammary susceptibility but ease of penetration of the teat duct by pathogens and accumulated previous infection (Andrews et al., 2004).

Factors such as climate, housing system, type of bedding and rainfall interact to the degree of exposure of teat ends to mastitis pathogens. Because dairy cow spend 40-65% of their life time lying down, the quality and management of housing for dairy cattle has a major influence on the type of mastitis pathogen that infect the mammary gland, as well as the degree of infection pressure (Radostitis et al., 2007).

Pathogenesis
The pathogenesis and characteristics of the disease are depends on factors in microbial ecology and the nature of pathogen and host defense (Jubb et al., 1993). Infections of the mammary gland always occur via the teat canal and the first impression of the development of inflammation after infection seems a natural sequence. However, the development of mastitis is more complex and expressed in term of invasion, infection and inflammation (Radostits et al., 2007).

After invasion the pathogen population established in the teat canal and a serious multiplication and extension in to mammary tissue may occur and result in the release of endotoxin, as in coliform mastitis which causes systemic effect. Inflammation follow infection and represent the stage at which clinical mastitis occur with vary degree of clinical abnormalities of udder and variable systemic effect from mild to peracute; gross and subclinical abnormalities of the udder include marked swelling increase warmth and, in acute and peracute stage, gangrene in some case and abscess formation and atrophy of the gland in chronic stage (Radostits et al., 2007). Trauma of the teat may cause breakdown of natural barrier and render the cow more susceptible to infection. Other resistance factors in addition to physical barrier that imposed in the teat canal include humoral and cellular component in the milk that inhibit microbial growth or enhance clearance of invading organism by phagocyte. Such substances are lactoferrin, immunoglobulin, lysozyme, and lactoperoxidase. Disturbance of any or all of this defense mechanism may increase susceptibility to mastitis (Benerjee et al., 2002).

Diagnosis
Clinical examination of the udder
The udder is first examined visually and then thorough palpation to detect possible fibrosis, inflammatory swelling, visible injury, tick infestation, atrophy of the tissue. The size and consistency of the mammary quarters are inspected for the presence any abnormalities such as disproportional symmetry, swelling firmness and blindness (Biffa et al., 2005). In addition the milk is examined for abnormalities such as clot, flakes, pus, malodorous milk, watery secretion, blood tinged milk etc. using a strip cup. Even though clinical examination of the udder is used for detection of clinical mastitis it is limited by its inability to detect subclinical mastitis (Radostits et al., 2007).

Screening test
The diagnosis of sub clinical mastitis has been made possible at the cow side through a number of screening tests such as California Mastitis Test (CMT), the N-Acetyl–B–D Glucosaminidase test (NaGase), direct microscopy
and electronic somatic cell count. These test are indirect methods for the detecting the presence of inflammation. They are unable to distinguish the infecting microorganisms, but rather determine the concentration of byproduct of inflammatory response (Radostits et al., 1994).

**California Mastitis Test (CMT)**

The CMT is the most reliable and inexpensive cow side test for the detection of subclinical mastitis. It is an indirect measure of cell count. The CMT reagent contain a detergent that react with DNA of the cell nuclei, and a PH indicator (Bromo cresol purple) that change the color when the milk pH increase above its normal value of approximately 6.6 (mastitis increase PH to 6.8 or above) (Radostits et al., 2007). This test is based on an increased number of leukocytes and increased alkalinity in milk due to mastitis (Chauhan and Agarwal, 2006).

Quinn et al (1999) has suggested that a squirt of milk from each quarter of the udder is placed in each of the four shallow cups in the CMT paddle and equal amount of commercial CMT reagent is added to each cup and gentle circular motion will be applied to the mixture, in horizontal plane and positive sample show gelling reaction within a few second. The reaction is a result of DNA extraction by dissolving the cell membrane of nuclei. According to Chauhan and Agarwal (2006) result read as follow:

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Description</th>
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<tbody>
<tr>
<td>Negative</td>
<td>Liquid milk with no streak or precipitation</td>
</tr>
<tr>
<td>+ (Weak positive)</td>
<td>Streaky fluid (cell count 500,000/ml)</td>
</tr>
<tr>
<td>++</td>
<td>Slimy</td>
</tr>
<tr>
<td>+++</td>
<td>Gelatinous</td>
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**Somatic Cell Count (SCC)**

There is a strong relationship between the SCC of the quarter samples of milk and the milk yield, with SCC increase slightly as milk production decrease but increasing markedly with intra-mammary infection of the quarter. The distribution of somatic cell count in a herd therefore reflects the distribution of intra-mammary infection (Radostits et al., 2007). Somatic cells are composed of leucocytes (75%) and epithelial cells (25%). Leucocytes (white blood cells) increase in milk in response to infection or injury while increase in epithelial cells is the result of infection or injury. The number of cells reflects the severity of mastitis (McDonald, 1997).

**White side test**

The principle of this test is based on the increased number of leukocyte in mastitic milk. In this test, 4-5 drops of test milk sample are placed on a clean dry glass slide. To this add a drop of 4% sodium hydroxide and mix with a glass rod. If the milk is from animal having mastitis, it becomes thickened and flakes appear. While the negative milk sample remain the same (Chauhan and Agarwal, 2006).

**Microbiological Diagnosis**

The advantage of a bacteriological examination is that the causative organism can be identified and provides a possibility for antimicrobial sensitivity testing (Andrews et al., 2004). Based on this method organisms causing bovine mastitis can be divided into five groups: gram-positive cocci, gram-negative bacteria (coliforms), Corynebacterium, Mycoplasma, and others, which include Nocardia, Prototheca, and yeasts (Sears et al., 1999). Isolation of organisms usually is performed by plating one standard loop (0.01 ml) of milk sample on the surface of blood agar plate (Hogan et al., 1999). A loop of milk sample was streaked on 5% sheep blood agar and the plates were incubated aerobically at 37 °C and examined after 24hrs of incubation for growth. The colonies were provisionally identified on the basis of staining reaction with Gram's stain, cellular morphology and hemolytic pattern on blood agar. The representative colonies were sub cultured on blood agar plate and on nutrient slants and incubated at 37 °C. The slants were preserved and maintained for characterizing the isolated by Slide Catalase test, KOH method for gram negative and gram positive bacteria (Andrews et al., 2004).

**Treatment**

The therapeutic and preventive effectiveness of antimicrobial drugs for bovine mastitis is dependent upon the etiological agent, proper use of the drug under consideration, dairy husbandry, sanitation procedure and the phase of the disease (Huber, 1994). When drugs are being selected for the treatment of subclincial mastitis, selection should be based on cost, safety, residue potential, distribution properties, and sensitivity data (Tyler, 1992).

In general, infections confined primarily to the milk and ducts (such as *C.bovis*, coagulase negative staphylococcus) are easily treated with intra-mammary antibiotics. In contrast, infections due to mastitis pathogens with systemic infection (such as *E. coli*, *K.pneumonia*, and *M.bovis*) are best treated with parental antibiotics. Mastitis pathogen that are the most difficult to treat are those that are principally infections of parenchyma tissue (such as *S.aureus*, *A.pyogens*); this is because it is more difficult to attain and maintain an effective antibiotic concentration at this anatomical site when administering antibiotics by the intra-mammary or
Staphylococci are commonly resistant to penicillin, streptomycin and tetracycline. However, usually effective antimicrobials include penicillinase resistant penicillin fluoroquinolones, chloramphenicol, erythromycin, cephalosporin, vancomycin, lincomycin and trimethoprim-sulfas are available (Hirsh and Zee, 1999). Bacteriological cure rate against S.aureus for antimicrobial therapy is relatively low due to pathogen characteristics such as the ability to survive inside the host cell and pathological change induced in chronic infections (Bramely, 1992).

Penicillin (intra-mammary) is effective for treating mastitis due to Streptococcus agalactiae and most other streptococci (Hirsh and Zee, 1999). Systemic therapy has also been reported to effective, but offers no clear medical or economical benefits over intra-mammary therapy (Tyler, 1992). Radostits et al (2007) has suggested that E.coli isolated from mammary gland of cattle is theoretically susceptible to ceftiofur, cefquinone, florquinolones, gentamycin, sulphonamide, oxytetracycline and trimethoprim.

Control
Successful mastitis control require a history of herd, identification of susceptibility pattern of the bacteria involved, as well as relevant information on milking management. Intra-mammary drug formulation is a suitable route for administration of long acting antimicrobial preparation at “dry off” is part of mastitis control program (Quinn et al., 1999). Many mastitis infections occur at the time of calving or preceding 1-2 weeks. A well drained pasture is preferred at calving area, with no access to ponds, swampy area or drainage ditches. A clover-grass sod is desired in contrast to muddy. Lots and pastures should be managed to prevent muddy areas where cattle would lie-down. Pens should be well bedded, clean, dry and comfortable (Jones et al., 1998).

The dry cow treatment with antibiotics showed a prophylactic benefit of 82% in reduction in the rate of new intra-mammary infections in the dry period and higher rate of eliminating infections than treating in lactation (Smith et al., 1997). Administration of intra-mammary antibiotics at the beginning of dry period is used for the treatment of mastitis caused by contagious pathogens, principally Staphylococcus aureus (Quinn et al., 2002)

Selenium and vitamin supplementation or injections at 2-3 weeks before expected calving have been shown to reduce mastitis after calving. However, vitamin-E levels of at least 1000 IU per day during the dry period and 500 IU per day during lactation were more beneficial than national research council’s recommended 100 IU per day (Weiss et al., 1997).

Radostits et al (2007) extensively reviewed the following as essential component of comprehensive udder health program: employ proper milking method, proper maintenance and use of milking equipment, dry cow management, appropriate therapy during lactation, cull chronically affected cows, maintenance of clean environment, monitoring udder health status, periodic review of udder health program and good record keeping.

References
Hirsh, D.C. and Zee, Y.C. (1999): Veterinary Microbiology. USA, Black well science, Inc. pp.115-249
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