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Emergence of Bovine Mycoplasmal Mastitis (BMM): Introspect on Disease, Epidemiological Pattern, Screening and Control Strategies

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Abstract

Owing to the intense and crash dairy cattle practices all over the world with an added effect of introduction of genetically improvised cow breeds, several mastitogenic agents including bacteria, fungi, algae and Nocardia are increasingly being incriminated in bovine mastitis. Mycoplasma being one of the agents that finds way into intramammary tissue, originating mainly from Respiratory and Urogenital infections in cattle. Mycoplasmal mastitis is of gravest concern of all causal types, since economic losses are tremendous. More over spread of the infection from one quarter to another and thereupon from one cow to another cow in a short duration in the form of mastitis storm is baffling the dairy producers and animal health managers. Diagnosis of this infection is cumbersome but bulk tank milk somatic cell counts have to be necessarily conducted in order to isolate the affected animals. Culture tests are difficult and have been replaced by the highly sensitive serological and molecular techniques such as ELISA, Immunoperoxidase and polymerase chain reaction for identification of the causal agent. Meticulous management practices based on substantial knowledge of the disease is considered instrumental in reducing the risk possibilities. Certain viral infections such as bovine parainfluenza and possibly bovine herpes virus need special attention to be kept off the bay, since these can potentially predispose the animals to Mycoplasma infection. The treatment of disease can be entailed but usually only in mild to sub-acute form where as Acute cases need to be culled. The problem that most effective mycoplasmocidal drugs with a protracted elimination from animal body has limited the scope of effective treatment of the mycoplasmal mastitis in dairy cows for the fear of xenobiotic hazard for human consumers. Though claims are piling up that Mycoplasmae have a zoonotic potential and can inflict disease on human consumers of milk, have not been substantiated by evidence based findings. Prophylactic immunization particularly against M.bovis in cattle has opened up a new gate way towards its effective prevention, if practiced on regular basis in endemically known places.

Keywords

Bovine mastitis; Mycoplasma; Epidemiological pattern; diagnostic trends; Mycoplasmocidal drugs

Introduction

Mastitis is one of the most important diseases with severe economic fallout in terms of production, cost of treatment and culling of animals. It affects 50% of herd population [1], with an estimated annual loss of Rs.6053.21 crores in India [2] and approximately 2 billion dollars in USA [3]. Under developed countries of south Asia have recorded prevalence of 51.3% mastitis in dairy cow herds [4]. The predominant cause of mastitis is microbial infection; different microorganisms elicit different responses of udder inflammation. The disease is incriminated with more than 137 pathogenic microbial species [5] and new agents being isolated every year from infected bovine udders including mycoplasmae, yeasts, fungi and algal agents owing to the organized and intensive dairy managemental practices [6].

Bovine mycoplasmal mastitis (BMM) is emergingly encountered in India during last more than one decade [7] and has gained increased medical and economic importance worldwide [8]. Increased incidence and the rapid spread of this infection among the herds are perhaps attributed to the fact that mycoplasmas of bovine origin have been increasingly incriminated in respiratory and genital infections. Unawareness and lack of proper diagnostic facilities has rendered veterinarians to be ignorant of this type of mastitis in India, more so under field conditions. Mycoplasmal mastitis also known as the contagious mastitis was first reported from England in1960 and has been recognized in USA since 1961.It has been reported from much

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of Europe, Canada, Japan, Australia and New Zealand. In India Contagious mastitis in bovine species has been reported by several researchers [9, 10, 11, 12].

Mycoplasmae are unique organisms which do not fit the description of a bacterium or a virus and are classified as microbes intermediate between the two, known as mollicutes. Mycoplasmae have the smallest size and smallest genome of 600-2200 kb [13]. Lack of a discrete cell wall makes them refractory to variety of conventional antibiotics that inhibit the cell wall synthesis in microbes. Mycoplasmas like most of bacteria are not intracellular but inflict disease extracellularly. Contagious mastitis in bovines due to Mycoplasma ssp. is characterized by rapid onset, involvement of all four quarters simultaneously marked drop in milk and a severe inflammatory reaction of udder with a mild systemic toxemia. What has added to the concern is that many mycoplasmae are reported to be potentially zoonotic particularly those incriminated in infections of food animals [14]. The animal products such as milk, meat and their bi- products may provide source of mycoplasma infection to humans [15].

Causal agents and Epidemiological pattern

Around 17 Mycoplasma spp. are incriminated in bovine intramammary infection (IMI) , most of them having been recorded in India [1]. The main causal agents are, M. bovirhinis, M.bovis, M. canadense and M. dispar, but M. bovis is considered as the chief agent, where as M. californicum and M. dispar are not prevalent in India [16]. However M. canadense and M.capricolum ss capripneumoniae have occasionally been isolated in India [17, 18].

Unfortunately not much is known about the factors that influence the frequency and distribution of this form of mastitis in dairy herds. Mycoplasma is classified as a contagious mastitis pathogen because the reservoir of the infection is in contact infected cattle, including calves. In contrary to other forms of contagious mastitis, mycoplasma infection can spread from the respiratory system to the udder. The spread can occur due to transmission through air. A history of respiratory disease or ear infections in calves occasionally precedes outbreaks of mycoplasma mastitis. The common source of infection is the purchase of cows, sub clinically affected with mycoplasma infection. Non-lactating animals are also at risk, as sub clinically infected carriers prior to freshening. After calving, the cows may never develop clinical mastitis, however may intermittently shed high levels of mycoplasma organisms in their milk [19]. Transmission between cows can occur during the milking process or through contamination of cow contact areas in the environment. The cows suffering from mycoplasmal vulvo-vaginitis and respiratory tract infection are at a higher risk of contracting mycoplasmal mastitis, besides young calves with sub clinical mycoplasmosis, play a significant role in transmission of infection to their dams [20]. Mycoplasma mastitis is reported to be most prevalent in geographical locations where an intense dairy cattle rearing is practiced [21]. The organism persists in unsanitary, warm, moist environment but destroyed by most disinfectants and teat dips. However, there is no evidence that environmental factors have any major influence on the course of disease, especially in the cases of mastitis and arthritis [22]. The agent is capable of persisting in the respiratory tract, where it remains infective as the young cows mature and can even be transmitted to the next generation. Moreover, there are possibilities for horizontal transmission at all stages of the development of cattle [23]. Seroprevalence surveys have revealed that highest rate of infection due to M.bovis was detected in cattle of 1-3 years age group which acted as potential source of infection to rest of the herd [24]. Mycoplasma and IBR virus are supposed to be associated as both have been detected from infected milk and the viral predisposition has been suggested [25]. Co-infection of M.bovis, P. multocida and M.haemolytica has also been recorded in some cattle herds [26]. Introduction of infected cows can spread the disease in a healthy dairy herd. The M. bovis causes depression of immunity and thus can survive the presence of large number of leukocytes in milk. Therefore suppression of cellular immune responses may be an important factor for the difficulty in control of this form of mastitis. Yet another factor, that makes mycoplasmae escape the host's immune mediated responses, is its capacity to undergo phase variation (rapid shift from antigen expression to non-expression and return) and also the ability to change the amino acid sequence and the cell mass [13]. The most common means of cow to cow infection takes place through milker's hands, the use of contaminated sponges or multiple use cloths for udder cleaning and use of ineffective teat dips. The bovine udder is infected upwards only through the teat canal, as demonstrated in a number of experimental infections [27]. Haematogenic or retrograde lymphogenic infection was also suggested, but has not been proved so far. The infected cows develop normal milk but still be sub clinically infected (and therefore able to shed the bacteria well into the next lactation). It is suggested that the rapid emergence of mycoplasmal mastitis might be related with the indiscriminate use of antibacterial drugs against bacterial mastitis, that render udder immune system dormant, particularly during periparturient and lactation stress . Intensive dairy cattle rearing and introduction of genetically modified cow breeds for enhanced productivity are other significant factors responsible for paving way to this form of mastitis.

Clinical Scenario

Contagious bovine mastitis caused by Mycoplasma spp.is highly suspected when the following clinical picture is present:

1.Failure of mastitis treatment with conventional antimicrobials and increase in mastitis cases in the herd [6].

2.There is usually precedence of infections causing Pneumonia,

arthritis, otitis, or vulvovaginitis some time back in the recent past in the herd [28].

3.Infection spreading from one quarter to another, such that all quarters become involved.

4.Severe loss of milk from affected quarters, with acute or sub acute clinical form.

5.Multiple quarters are involved and there is dramatically decreased milk production • Cows appear otherwise healthy but have severe mastitis • Milk has sandy or flaky sediments in watery or serous fluid. Milk from affected quarters is tannish or brownish, ultimately becoming watery, laden with pus and flaky debris [29].

6.Systemic sign of fever co-associated with varying inappetance and a toxemic reaction in acute cases.

7.In some instances, the Mycoplasma mastitis outbreak may be transiently associated with increased cases of arthritis or pneumonia or both [30].

Laboratory Confirmation

1. Direct culture examination

Mycoplasma can be isolated from milk samples by direct culture on special media (PPLO agar) enriched with horse serum. However the limitation with the procedure is the duration (7-10 days) of incubation. Special condition of reduced oxygen tension is also required. Identification is based on film formation, Diene's stain and inhibition of fried egg mycoplasma culture growth by the use of specific hyper immune sera on the PPLO plates. Thus added expense and the primary specificity to distinguish between the true pathogens and the commonsels. Culture of composite milk samples (one sample containing milk from all the quarters) or bulk tank milk samples has been advocated as a primary screening method to determine the mycoplasma status of a herd [21]. However, bulk milk culture examination may not detect the cows excreting pathogens below the threshold level and that the shedding of organisms is inconsistent in dairy cattle with intramammary infection, increasing the risk of misdiagnosis, if multiple samples are not tested [31].

2. Bulk tank somatic cell count (BTSCC)

It increases in all cases of mycoplasmal mastitis [32]. A cell count of 5×105/ml or above should be considered abnormal. However somatic cell count of suspected milk samples is non-specific and is generally considered as mastitis index in intramammary infections, irrespective of the type of invading mastitogens. The IDF criterion (5×105/ml) for somatic cell count of mastitic milk is same for bacterial, mycotic and mycoplasmal mastitis. Therefore increased somatic cell count of suspected milk samples should have a positive correlation with that of culture findings, in order to eliminate the possibility of non-mycoplasmal causes of mastitis. In a pathogen free milk herd, age of the cow, production level and stage of lactation, significantly influence SCC [33]. Several cows showing pathogen free milk samples might be having abnormally high SCC [34], because of udder affections other than infection.

3. Serological diagnosis

Based on monoclonal blocking ELISA and improved immuno-binding test for detection of antibody (IgM and IgG) to M.bovis have proved to be highly specific and sensitive with no cross reaction [35, 36]. These tests are conducted for screening of a herd when the carrier status is to be investigated, besides clinical diagnosis.

4. Molecular diagnosis

Based on specific DNA probe for the detection of M.bovis and Polymerase chain reaction for the detection of M.bovis antigen from bovine milk and mucosal samples was found positively correlated with that of B ELISA [37, 38]. Since culturing mycoplasmas is so demanding, PCR-based assays for diagnosis of infections have been developed. Although these are rapid and more sensitive than culture-based methods, interpretation of the results can be difficult. PCR may detect DNA from non-viable cells and also from mycoplasmas that have colonized, but not infected, the patient. Nevertheless, PCR assays for mycoplasmas, mainly M.bovis, and M. pneumoniae, have been developed commercially. However, PCR-based mycoplasma detection methods are still not widely used in clinical laboratories however PCR method used as a four-way multiplex real time assay including primers for general Mycoplasma spp., Mycoplasma bovis, Mycoplasma bovigenitalium, detected (60.3%) classified as Mycoplasma bovis, (27.6%) as Mycoplasma ovigenitalium, and (12.1%) were only detected by the Mycoplasma spp. primers. Direct PCR amplification of a broth enriched milk sample can rapidly detect Mycoplasma spp. in bulk tank and pen samples, and can give results comparable to conventional culture methods. PCR also offers the possibility of providing species differentiation of positive samples [39].

Pulsed-field gel electrophoresis (PFGE)

It is performed on mammary and non-mammary isolates identified as the similar strains of mycoplasmae [31].
Control strategies

1. Chemotherapeutic management:

Lack of a definite cell membrane makes Mycoplasma spp. refractory to majority of antibacterial drugs that inhibit cell wall synthesis. However the drugs that inhibit DNA synthesis have a role as mycoplasmostatics if drug resistance is not a

problem. Intramammary infusion of antimycoplasmal drugs has fair chance of healing in positive cases. However Metaphylactic drug therapy should form the basis of treatment of this form of mastitis [30]. Enrofloxacin was found to be the drug with highest therapeutic efficacy against Caprine mycoplasmosis in one experimental study [40].

Other quinolones, showing in-vitro activity against M.bovis isolates are danofloxacin and marbofloxacin [41]. Amino glycoside drugs such as tiamulin, and lincomycin were also shown to have effective elimination of several caprine Mycoplasma spp.[40]. The M.bovis isolates were found highly susceptible to tiamulin in-vitro [41]. However tiamulin hydrogen fumerate is yet to be licensed for treatment in cattle owing to its xenobiotic effect on human health. The M.bovis has been found resistant to drugs like tilmicosin, tetracycline and spectinomycin, traditionally used for their control [42]. However oxytetracycline and roletetracycline are reported to be effective in animal mycoplasmosis with good clinical recovery [43, 40]. Oxytetracycline was found to be successful in imparting clinical recovery of cows with late post parturient sub acute mastitis, that poorly responded to amoxicillin and cloxacillin for one week followed by gentamicin for next one week and then to collistin sulphate intramammary infusion [44]. Interestingly the milk samples were found to be sterile prior to treatment, on common bacteriological media, as Mycoplasma Spp. require special enrichment media and laboratory culture environment. Pertinently, the milk was found negative for mycotic culture, also. However in one study 50% M.bovis isolates were found to be resistant to oxytetracycline in-vitro [45].

Tulathromycin has been reported effective in the treatment of calf mycoplasmosis with the limitation that it is yet to be licensed for the use in cattle over 20 months age owing to its excretion through milk,since the single dose of this drug maintains 7-14 days therapeutic blood levels [46]. Tulathromycin in one study, was found to be the most effective chemotherapeutic agent [47].Treatment regimes of tylosin and oxytetracycline, independantly, eliminated acute mycoplasma mastitis infection in ewes due to M. Californicum, where as concurrent use of intramammary and intramuscular tiamulin failed to do so [49].

2.Prevention

The important aspect of prevention of intramammary mycoplasmosis focuses on, thorough screening of animals before they enter the herd. Requesting cultures for Mycoplasma on the individual cow or from bulk milk tank samples before purchasing new cows is highly recommended. Three to five sam¬ples should culture negative for Mycoplasma over the 1 to 2 months prior to adding new animal to the herd. Although this seems like a lot of testing, the con¬sequences of introducing Mycoplasma into a dairy herd can be disastrous. The source of the cow introductions should be considered in determining the number of cultures performed. Pregnant heifers can carry Myco¬plasma in their udders and become clinically infected after they calve. Therefore, heifers should also be cultured. Mastitis bio-security programme can be used to decrease the risk of purchasing infected cattle.

Potential interventions, such as therapeutics, vaccines, or management control measures, are supposed to be most effective before 50 days of age based upon the cumulative incidence of colonization in animals [26].

Herds in an endemic area should be routinely monitored through bulk milk culture. This should be done at least monthly, and more often in previously affected herds. Maintain a closed herd if possible, however if it is necessary to purchase new cows, it should be ascertained that these are culture negative, as even a single positive cow can pose the threat of " mastitis storm", in the herd [29]. Most transfer of mycoplasmal infection within herds occurs at milking by means of fomites. Many new herd infections occur from the introduction of replacements with infected udders. Mycoplasmal mastitis, however, may occur in previously clean herds without introduction of new animals or history of previous intramammary treatment [28].

The Package of practices

Control measures to be adopted when a herd is mycoplasma mastitis positive include the following:

• Culture all cows for mycoplasma.This is done on composite samples i.e. milk from all the four quarters.

• Milk samples of 20 cows can be considered in a single culture and if found positive, the individual cows should be screened thereof to identify the infected cows.

• Sanitation is extremely important during collection of milk samples for culture. The cow's udder, feet and lower legs must be clean. The udder and teats should be disinfected with a new alcohol pledget immediately prior to sampling.

• Remove all cows with positive mycoplasma cultures from the milking strings of the herd. These cowsshould be under taken for regular treatment with a suitable mycoplasmocidal drug; however the severely affected clinical cases are recommended for culling and slaughter because treatment in such cases is disgusting [25].

• Sub-clinically infected cows should be segregated and maintained separately on the dairy farm; always milked last and treated. The cows which are not culture negative post treatment should not be allowed to re- enter the mycoplasma free herd.

• It is always better to dry off the infected cows and place in a separate dry cow herd. After dry cow Therapy, resampling should be done at least twice and removal of positive cases at the time of freshening should be ensured.

• Monitor the herd by tank milk sampling after each string is milked once each week, until negative cultures are obtained two times from all samples. Removal of all positive cows from the milking strings has to be ensured.

• Teat dipping with an effective teat dip should be rigorously followed with provision of sand bedding instead of straw bedding [49].

• Calves are infected with mycoplasmas by contact with the dam at birth, or ingestion of contaminated discard milk. After assuring that all discard milk used as feed is pasteurized, calves can be treated with appropriate antibiotics by injectable and/or oral route to provide medication coverage for 10 days. All calves at risk should be treated to reduce mycoplasma burden on the premises [50].

• In calf operations that have multiple sources of infection, it has been found useful to apply multistrain autogenous bacterins in three to four weekly doses starting soon after birth. For complete suppression of pneumonia, calves will need to be treated as indicated above as supplement to the vaccination programme.

• Heifer calves can benefit from vaccination with commercial bacterins several weeks prior to transportation to dairies, and revaccination during dry periods is a recommended follow-up [50].

Vaccination

Though vaccination of M.bovis is not completely effective, but, it can affect the course and severity of infection in vaccinated cows [51]. However others have reported that the vaccination was not efficacious to prevent, decrease the incidence or ameliorate the clinical signs of mycoplasmal mastitis [28]. Vaccination of dry cows has not been shown to prevent infections, although it may not have negative effects [28]. An autogenous bacterin made from the mycoplasma isolated from clinical cases of mastitis, after giving two doses of vaccine, the number of new cases of mycoplasma mastitis was shown to drop significantly [51]. Mycoplasma bovis vaccine has been found to be first line defense to overcome pathological devastation due to its respiratory form in calves under controlled setting but under natural field conditions it has been evidenced to be unrewarding [30]; although its efficacy remains to be obscure in protection against mastitis in dairy cows, though the vaccine has been reported to be effective by some workers[53]. In another trial the vaccinated cows produced high immune response with negative culture findings and PCR, indicating that the prepared vaccine was able to protect the animals against M. bovis infection [54]. However calves in problem herds should be vaccinated with M.bovis strain, that would influence reduction in mastitis cases, since one of the sources of infection appears to be the calves with respiratory form of the disease. One of the possible reasons for poor response to immunization in the prevention of mycoplasmal mastitis may be likely due to the fact that the disease is a multietiological one and the commercial polyvalent vaccine being not available [55]; the causal agent possesses ability of rapid shift from antigen expression to non-expression and return as a result the polyvalent vaccine has not met success.

In situations where a low number of cows culture posi¬tive, it is recommended to cull all positive cows. Replace¬ment animal numbers, farm finances, stocking density, and expansion plans are some of the factors to consider when determining if culling all positive cows is feasible.

Conclusion and Recommendations

Mycoplasmal mastitis is emergingly encompassed all over the world including India, as a consequence to crash and intensive dairying for the want of more milk production, in developing countries. Genetic improvisation of milk producing cows has yet added to their susceptibility and more incidence of the disease. Infectious bovine rhinotracheitis (IBR) infection which is nowadays reported endemic in several places has a strong possibility of causing mycoplasma mastitis as co-infection in susceptible herds; it needs to be addressed on war footing. Introduction of unscreened cows or calves to the herds underlines the main source of transmission of infection. It is a routine practice in several developing countries that newly purchased cows are introduced to the dairy farms/ herds unscreened. Prior testing of new cows requires culture examination of milk at least three times.

Of all strains, M.bovis is the major agent of bovine mastitis, which is at the same time responsible for respiratory disease and arthritis in young calves that could contribute significantly to the spread of infection to their dams in problem herds. Thus vaccination with M.bovis in calves would help reduce the incidence in a given herd. Several antimicrobial drugs are effective in the elimination of all stages of mycoplasmal infection, but what is of serious concern is that these antimycoplasmal drugs are not safe in lactating animals thus all of these are not licensed to be used. However oxytetracycline, tiamulin and tulathromycin if administered for the treatment of mycoplasmosis in cows, the milk has to be discarded for at least till 72 hours post treatment, being unfit for human consumption. The immunization with M.bovis vaccine has not yielded encouraging results as it alters only the clinical severity and course of the disease. Therefore vaccine has a fair chance of reducing the clinical sickness and giving it a try in problem herds is not associated with deleterious effects, anyway. Mastitis cases refractory to conventional antimicrobial therapy under field conditions and out breaks of mastitis in a given herd are two important factors that should arouse strong suspicion of mycoplasmal infection. Mastitis screening especially bulk tank milk cultures once in three months is mandatory for all dairy farms and even small dairy units in order to ascertain the

mycoplasma status of the udder. Three times culturally positive (at monthly interval) cases must be isolated, treated in separate wards and milked last. Clinically acute cases have no good prognosis and thus need to be culled. The milk of affected cows has to be boiled at 100°C for several minutes prior to feeding to neonatal calves.

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