**Mastitis Management in Dairy Animals**

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Preface

India ranks first in the world in milk production with an estimated 127.3 million tonnes in 2011-12. The demand of milk by the year 2030 would be about 200 million tonnes, which would imply an annual increase of around 4 million tonnes during the next two decades. Milk is vital part of human food and is an important item of the food table of majority of people in India. It is also a major cash commodity for millions of farmers. Any circumstance leading to decrease in milk quantity and quality will therefore have a strong bearing on livelihood and household nutrition. Moreover, national average milk yield and quality are low. One of the major reasons for both low yield and poor quality of milk is mastitis, which ranks first among the diseases causing substantial loss to the dairy farmers. Over the decades, its prevalence in dairy animals has increased alarmingly. The recent trend of transformation from traditional dairying towards commercial dairying where hundreds to thousands of animals are reared together necessitates development of suitable measures to control mastitis. The adverse impact of mastitis on smallholder production systems is also very high. Mastitis management thus assumes high significance not only to curtail the losses associated with low production but most significantly to the human health related issues. Due to problems in detecting subclinical mastitis, the milk enters into the milk procurement system, and as a consequence, the pathogens and their toxins in the milk may pose health problems to the consumers.

The National Academy of Agricultural Sciences organized a Brainstorming Session on 'Mastitis Management in Dairy Animals' to strengthen the research on diagnosis, management, development of mastitis bio-markers, vaccines and to design a strategy for effective mastitis prevention and control. Forty five distinguished invitees including leading experts participated in the Session. A set of policy measures and recommendations has emerged out of the deliberations. I am optimistic that this Policy Paper would signal a paradigm shift in the bovine mastitis management. The Academy is grateful to Dr. A.K. Srivastava, Director, NDRI & Convener for successfully convening the Session and to all the distinguished participants for their active participation. Grateful thanks are due to the Resource Persons, the Reviewers and the Editors of the Policy Paper.

(R.B. Singh)
President, NAAS
Mastitis Management in Dairy Animals

1. PREAMBLE

India ranks first in the world in milk production and dairying in India is a classic example of production by masses rather than mass production. Due to changes in human food consumption patterns, demands for fruits, vegetables, milk and milk products, meat, poultry and fisheries have been increasing over the period in recent years. Among the different food sectors, the growth in dairy sector has been commendable. It was not a simple task to increase the milk production from 17 million tonnes in 1950-51 to an estimated 127.3 million tonnes in 2011-12; it is projected that the milk production will touch 133 million in 2012-13. Increase in milk production boosted the per capita availability of milk in the country. In 1950-51, the per capita milk availability was only 130 gram per day and today the national average is just above the ICMR recommended level of 280 gram per day and is expected to reach 290 gram per day in 2013. The rate of growth in milk production in India is also substantially higher (3.6 per cent) than the world average of 1.5 per cent. However, the total projected demand of milk by the year 2030 would be about 200 million tonnes, depending on assumptions about income, population, urban growth, and expenditure elasticity parameters, which would imply an annual increase of around 4 million tonnes during the next two decades [1]. At the existing rate of growth in milk production, supply is likely to fall short of the demand in next ten years. Among the several barriers in achieving the production targets, mastitis continues to remain as a challenging impediment, since the affected quarters may have 30 per cent less productivity and cow may lose about 15 per cent production [2].

Mastitis in dairy animals is considered as one of the most important economic diseases resulting into huge economic loss to the country. Globally, mastitis accounts for about 38 per cent of the total direct costs of the common production diseases [3]. In India, the economic losses due to mastitis have increased about 115 folds in last five decades [4]. Lack of awareness, delay in detection of sub-clinical mastitis, lack of markers for detecting ensuing mastitis, unhygienic milking practices, diverse production systems, inadequate treatment etc. are some of the important contributing factors in higher incidence of mastitis. We need to bring strategic interventions to prevent and control mastitis for sustaining milk production. Since mastitis affects the milk quality, its consequences are not restricted only to but beyond the dairy farm. Increasing concerns among the consumers about the antimicrobial residues,
antimicrobial resistance, milk quality and animal welfare further demand proper policies for an effective prevention and control of mastitis.

The loss of milk production is not just restricted during the course of the disease but may continue throughout the life of the animal because of the permanent damage that mastitis can cause to the mammary secretary tissues. The milk from the suffering animal generally carries microbial load that renders it unsuitable for human consumption. There is a considerable increase in the somatic cell count in milk of cows and buffaloes suffering from mastitis. It is, therefore, important that India undertakes a nationwide plan to prevent and control mastitis.

Keeping in view the alarming situation of mastitis amongst productive animals, National Academy of Agricultural Sciences (NAAS) organized a brainstorming session on “Mastitis Management in Dairy Animals” on 31st October 2012 to gauge the status of mastitis and evolve suitable national strategies for its effective prevention and control.

2. MASTITIS SCENARIO (EPIDEMIOLOGICAL STATUS): GLOBAL VIS-À-VIS INDIA

With the increase in milk production, the incidence of mastitis has also increased. Surveys on the prevalence of mastitis in most of the countries, irrespective of the cause, show a comparable figure of 50 per cent among dairy cows [2]. Subclinical mastitis which is believed to be more prevalent rather than clinical in most countries ranged from 19 to 78 per cent [5]. Although controlled studies involving large sample sizes are very few in the country, the available reports suggest same pattern. Analysis of 513, 1707 and 1115 lactation records of Sahiwal and crossbred cows, and Murrah buffaloes, respectively in an organized farm in northern India over a period of 9 years revealed that overall incidence of mastitis was 13 per cent with significant difference between the breeds. Sahiwal cows had higher incidence (20.66 per cent) compared to crossbred cows (14.18 per cent) or Murrah buffaloes (7.44 per cent). An influence of season on disease incidence was also observed in both cows and buffaloes in the same study.

In other studies, it has been shown that the incidence was the highest among pure-bred Holsteins and Jerseys but the lowest in local cattle and buffaloes. In Haryana and Rajasthan, the prevalence has been reported to be 36.69 per cent and 60.25 per cent, respectively [6]. In several studies, it has been reported that subclinical mastitis was 15 to 40 times more prevalent than the clinical form and was of longer duration, difficult to detect, adversely affected milk quality and
quantity. It constitutes a reservoir of microorganisms that lead to cross-infection of other animals within the herd.

Based on the published reports, it is evident that the average prevalence of mastitis in 1960s to early 1990s, was not more than 30 per cent but increased afterwards to even more than 60 per cent [7]. Two decades ago, the mean incidence of clinical mastitis in India was 1-10 per cent with subclinical mastitis ranging from 10 50 per cent in cows and 5-20 per cent in buffaloes, while recent studies showed higher incidence of subclinical mastitis ranging from 20 to 83 per cent in cows and 45 per cent in buffaloes [8]. Analysis of the data from more than 100 recent studies spread over 21 States of India indicate that the overall prevalence of mastitis ranged from 25 to 97 per cent with a mean prevalence of about 50 per cent [8]. This clearly indicates the drastic increase in the prevalence of mastitis especially the subclinical form of the disease, which is an alarming situation for the dairy sector in the country.

3. CHANGING EPIDEMIOLOGY

Mastitis is the outcome of interaction of various factors associated with the host, pathogen(s) and the environment. Association of some host, and managerial and housing determinants with mastitis is well established. At least 137 species of microorganisms from a broad phylogenetic spectrum, including bacteria, yeast, fungi and algae, are able to cause bovine mastitis. However, amongst these, only 5 species of bacteria account for the bulk of bovine mastitis cases [9] but dominant causal agents may have some geographical signatures, as the distribution of pathogenic bacteria displays a substantial geographic variation.

Causal pathogens can be divided into two groups based on their source: environmental pathogens and contagious pathogens. Coliform organisms (Escherichia coli, Klebsiella sp etc.) and streptococcal organisms (Streptococcus uberis, S. bovis and S. dysgalactiae) are the important environmental pathogens. The major pathogens involved in mastitis are Streptococcus agalactiae, Staphylococcus aureus, Corynebacterium bovis and Mycoplasma spp. The distribution of pathogens varies among countries and even within country, production systems, farms and individual animals. For example, Staphylococcus aureus is most frequently encountered in clinical mastitis, followed by Streptococcus dysgalactiae in Norway [10] while in midwestern United States, coliforms are the most frequently isolated bacteria. In Europe, clinical Klebsiella mastitis occurs less frequently than clinical E. coli mastitis. In contrast, coliforms are less important and Streptococcus uberis is the main concern in both clinical and subclinical mastitis in New Zealand [11].
In India, *Staphylococcus* spp. have been reported to be the main etiological agents of mastitis in cattle and buffaloes. However, there are no studies on nationwide distribution of mastitis-causing bacteria in India. Apart from regional differences, cows in tie-stalls have higher incidence of *Staphylococcus aureus*, *Streptococcus uberis*, coagulase-negative staphylococci and other streptococcal infections compared to those in free-stalls, where *Klebsiella* sp. and *E. coli* are main concerns [12]. Collectively, it suggests that distribution of organisms may vary between regions and husbandry systems and it is important to pre-ascertain the epidemiological pattern of mastitis pathogens in the implementation of management strategies.

In recent years, there have been changes in the relative and absolute importance of different pathogens. In UK, during 1960s, it was observed that *Staphylococcus aureus* was the most common organism in mastitis, but in 1980s, *E. coli* was most commonly isolated from the milk of mastitis affected cows and the same trend was also continued in 1990s. In several countries, *S. aureus* continues to be the major cause of sub-clinical mastitis and the pathogens previously considered to be purely environmental may also be capable of causing persistent infection.

The major objectives of the epidemiological investigations include the identification of risk factors at farm level, major pathogen(s) involved and the susceptibility of the host. India being a large and diversified country with different farming systems and agro climatic conditions, the prevalence of mastitis and the pathogen(s) involved are likely to vary with places and herds. Hence, obtaining the ground situation of the disease and characterizations of epidemiological parameters to be intervened are of paramount importance.

### 4. LOSSES DUE TO MASTITIS

The losses are either due to temporary or permanent loss of milk production, poor milk quality, discarding of milk from affected animals prior to or after antibiotic treatment and pre-mature culling of the cow or reduced productive life of animals. The loss due to subclinical mastitis overweighs the loss associated with clinical mastitis.

In the affected animals, the milk yield is reduced considerably. Estimates of milk yield loss by different workers range from 100 to 500 kg/cow per lactation. When clinical mastitis occurs, additional costs result from discard of abnormal milk, cost of drugs and veterinary services. According to a study, the estimated loss following clinical mastitis in cows was almost 700 kg in first lactation and 1,200 kg in the second or higher lactation [13]. Several studies conducted at the United States show that costs related to mastitis on dairy farms are approximately U$ 200 per cow/year. This gives an annual loss of 2 billion dollars for dairy industry [14]. It is generally agreed
that at least 70 per cent of economic loss is due to reduction in milk production and discard of milk from sick animals. Other causes are the elimination of milk containing residues of antibiotics used in treating sick animals, loss of genetic stock by culling cows early and therefore more expensive replacement, veterinary fees, cost of medicines and payment of extra labour hours [7].

In India, annual economic loss incurred by dairy industry on account of udder infections is estimated to be Rs. 6053.21 crores and out of which loss of Rs. 4365.32 crore (70 - 80 per cent) has been attributed to sub-clinical mastitis [4]. In another report from India, the annual economic loss due to mastitis has been calculated to be Rs. 7165.51 crores; losses being almost same for cows (3649.56 crores) and buffaloes (3515.95 crores). Subclinical mastitis has been estimated to account for 57.93 per cent (4151.16 crores) of the total economic loss due to mastitis [15].

5. HARMFUL EFFECTS BEYOND UDDER

Earlier, it was thought that the effect of mastitis was restricted to udder only but now it has been proved that mastitis affects the reproduction efficiency of the animals also, especially during early lactation period. Extensive studies indicated that both clinical and sub-clinical mastitis alter the reproductive process at several levels. Mastitis delays the postpartum ovarian function and alters some of the key reproductive functions like ovulation, fertilization, implantation, and pregnancy maintenance. Acute mastitis delays the calving to first service interval, calving to conception interval and increase the number of services per conception. When clinical mastitis occurs before the first artificial insemination (AI), calving to first service interval is significantly increased, compared to when it occurs after the first AI [16].

Bacterial toxins released during mastitis influence conception and early embryonic survival in affected cattle by stimulating the production of prostaglandin F₂α, which subsequently causes luteal regression, thus potentially causing the loss of an established pregnancy [17]. Mastitis leads to the production of bioactive molecules in the reproductive tract tissues. For example, *E. coli* endotoxin does not usually penetrate from the udder into the blood, but can induce massive release of cytokines. These cytokine-mediated neural and endocrine changes play a key role in the inflammatory process. Clinical mastitis also induces hormonal alterations like decreased pulsatile secretion of luteinizing hormone (LH), significant decrease in the ovulatory LH peak, decreased estradiol production leading to decreased estrus expression and failure of ovulation [18].

It has been reported that the probability of conception decreased by 44 per cent when mastitis occurred a week before insemination, by 73 per cent when it occurred
during the week of insemination, and by 52 per cent when mastitis occurred during the week after insemination. The effect of mastitis is not only limited to the affected animals but also continues to the developing fetus, since the daughters born to the cows that suffered mastitis during gestation had reduced reproductive efficiency. Mastitis in pregnant cows could decrease the number of healthy follicles in the developing fetus and compromise future fertility. Anti-Mullerian hormone, a reliable biomarker for potential fertility, is severely decreased in the developing fetus as the number of mastitis events during gestation of their dams increases [19].

6. DIAGNOSIS

Traditionally, detection of mastitis is carried out by estimation of somatic cell counts, estimation of biomarkers associated with the onset of the disease (e.g. the enzymes N-acetyl-β-D-glucosaminidase and lactate dehydrogenase), isolation, identification and characterization of the causative agent by culturing [20]. Since, these methods have certain limitations, there is an urgent need to develop a rapid, specific, sensitive and reliable diagnostic test suitable under field conditions where sophisticated instruments are not available. Unlike in developed countries, where automated systems of dairying are practiced in which screening of animals for milk somatic cell count is a routine practice, in our country it is not possible owing to smaller dairy units.

Among the several aids available to diagnose mastitis, alterations in milk quality in terms of Somatic Cell Count (SCC), Electrical Conductivity (EC) and pH have been shown to be strongly associated with mastitis. Thus, assessment of these parameters has been employed to detect subclinical mastitis in smaller dairy units. However, the standards of these parameters to detect subclinical mastitis have been developed elsewhere in highly organized farms but the suitability, practicability, sensitivity and specificity of these standards in unorganized sectors have not been studied in detail. Inspite of having significant contribution from unorganized dairy farms in milk production, no concerted effort has been made to apply these tests and standards to check the milk quality in the small holder production system.

The development of tools to detect “ongoing” mastitis in a cow at the “cow side” is the most suitable option to reduce the loss due to this disease. Recently, significant advances have been made in identifying nucleic acid markers along with other novel biomarkers associated with mastitis and combinations of conventional tests in sensor based platforms for accurate identification of mastitis. Proteomic studies on milk from mastitis affected and normal animals, although started recently, are needed urgently in detailed way to identify mastitis specific markers. Once such protein markers are identified, these can be translated into effective and easy to use “on site” test kits
using immunoassay methods. With the advances in proteomics and genomics now, development of such kits is possible.

In large scale dairying, development of novel analytical platform incorporating enzymatic assays, immune assays, biosensors and nucleic acid tests could be a viable option. Also, development / standardization of “electronic tongue or nose” that could differentiate the mastitis milk based on organic and inorganic cations and anions, and volatile substances would be a reliable and sensitive method to be applicable in automated milking farms.

7. PREVENTION AND TREATMENT

The use of vaccination to control infectious diseases in dairy cattle is common and vaccination against mastitis causing pathogens is a control strategy used by some dairy farmers in developed countries. In fact, commercial vaccines against mastitis caused by *Staphylococcus aureus* and *E. coli* are available in USA. These vaccines are said to reduce the severity and duration of clinical mastitis as well as to provide a degree of protection against new intra-mammary infection by these pathogens. The J-5 type of vaccine is being used to protect against intra mammary infection caused by coliform (*E. coli, Klebsiella species, Citrobacter sp, and Enterobacter sp*). When given to adult cows during the dry period, it has been shown to reduce the incidence of coliform mastitis. Since the coliform vaccine has been shown to reduce population loss of the related herds, majority of consultants recommend their use in most of the dairy herds.

Several experimental and field trials have shown that commercially available *Staphylococcus aureus* vaccines resulted in increased cure rate of mastitis and thus reduce the development of chronic infections [21]. However, it is a well known fact that the successful control of *Staphylococcus aureus* mastitis depends on prevention of new infections rather than reducing development of chronic infections. Therefore, the failure to prevent new infections is probably the reason that this vaccine is being used on a limited basis in mastitis control programs. Since there are no vaccines commercially available to protect against *Streptococcus* mastitis, the research for such vaccines has been initiated in recent years. Further, it is necessary to identify different causal bacterial phenotypes alongwith characterization of their genotypes in different agro-climatic areas in order to deploy such vaccines. At present, in India, no vaccine is available against any of the major mastitis causing organisms, although, some efforts were made in the past.

Despite improvement in understanding the patho-physiology of mastitis, successful treatment strategies for all organisms have not been achieved. The main aim of the
treatment of clinical mastitis has been to restore the milk yield with short duration of treatment rather than eliminating the infection, which could be one of the probable reasons for limited success in mastitis control or reoccurrence of mastitis despite the treatment, especially under Indian conditions. Existing reports indicate that 20 per cent of the mastitis affected animals relapsed by 2.36 times in same lactation, suggesting failure to achieve pathogen clearance with the antimicrobials used or resistance of the underlying organism to the antimicrobials used. Further, different groups or species of organisms need different treatment strategies and thus, the therapeutic strategy should be based on organism culture and anti-microbial sensitivity results. However, it is uncertain whether result of “time consuming” antimicrobial sensitivity test can accurately predict the success of treatment as most drugs effective in vitro often fail to cure the animals. Although antibiotic therapy continues to be an important part of current mastitis control programme, alternate approaches are needed for reasons as explained above. Differential efficacy of intra-mammary drug infusions during lactation period particularly against major mastitis pathogens, cost-effectiveness of such treatments, potential danger of antibiotic residues, emergence of antibiotic resistant human pathogens and increased public awareness on food safety are among most important considerations for non-antibiotic approaches for mastitis therapy.

8. MASTITIS CONTROL PROGRAMS

In UK and EU, the prevalence of mastitis has been brought down significantly either by adopting five-point or ten-point plans. The five point plan or five-pronged approach comprises of (i) rapid identification and treatment of clinical cases, (ii) routine whole herd antibiotic dry cow therapy, (iii) post-milking teat disinfection, (iv) culling of chronically affected cows and (v) the routine maintenance of milking machine. At the end of the last century, the National Mastitis Council of USA and Canada expanded the five-point plan to a ten-point plan with 73 sub-points. The ten points are: (a) establishment of goals for udder health; (b) maintenance of a clean, dry and comfortable environment; (c) proper milking procedures; (d) proper maintenance and use of milking equipment; (e) good record keeping; (f) appropriate management of clinical mastitis during lactation; (g) effective dry cow management; (h) maintenance of bio-security for contagious pathogens and culling of incurable and chronically infected cows; (i) regular monitoring of udder health status; and (j) periodic review of the mastitis control program [20].

After detailed deliberations on epidemiology, etiology, public health significance, udder immunity, bio-markers identification, antibacterial and herbal treatment for mastitis, the
brainstorming session concluded that the following measures are essential towards control of mastitis in dairy animals.

9. POLICY MEASURES

◆ Establishment of a separate body in India on the lines of National Mastitis Council in USA and Canada to formulate, facilitate and coordinate research and development to control the disease.

◆ Dairy cooperatives should come forward to establish and promote the concept of "Clean Milk" and provide incentives in the form of better price for clean milk to the farmers. Testing Centres at various levels need to be established for screening of milk for somatic cell count and total bacterial count.

◆ Milk quality standards need to be revised and a check system to be applied for the prevention of sale of substandard milk as mastitis influences milk quality and in turn adversely affects the consumer health.

10. RECOMMENDATIONS

1. Priority should be given to study the impact of mastitis on smallholder production systems as it may undermine the household nutrition of already undernourished mostly poor rural population on one hand and the socioeconomic conditions of the farmers on the other.

2. The roadmap for mastitis management should be devised from the perspectives of the three most important stakeholders, viz, the dairy sector; the farmer and the consumer.

3. Mapping (both temporal and spatial) of mastitis should be done across the country and also to initiate research on identification of genome-based bio-marker linked with subclinical mastitis. Species and breed variation in susceptibility or resistance to mastitis is also need to be assessed in detail.

4. In the absence of true profile of the causative organisms, the potential role of many microbes involved in mastitis might get ignored, which is otherwise essential for effective therapeutic intervention. Therefore, meta-genomic approach is essential to study on intra-mammary microbial community in healthy and mastitis affected animals for better understanding of the pathogenesis.

5. In the wake of bacterial resistance to antibiotics and the associated problems of drug residues in milk, threatening consumer health, indiscriminate use of
antibiotics as a therapy for Mastitis should be checked. Strict monitoring is required for adherence to recommended milk withdrawal period following the antibiotic therapy.

6. A shift in research towards alternative methods of disease control (immune modulation and nutritional support) for enhancing udder immunity is required. Government should provision adequate funds and infrastructure required to undertake research in this direction. A network programme on "mastitis control" may be taken up by the Indian Council of Agricultural Research along with State Agricultural Universities and other related agencies.

7. A comprehensive package about disease awareness, management and control measures is to be developed for education at farmers' level as udder health, hygiene and nutrition play an important role in the control of mastitis. The best practices for reducing the incidence of mastitis like teat dip after milking; not allowing the cows to sit for 30 minutes after milking should be propagated widely among the farming community through mass media.

8. Effective organization of veterinary services with periodical training of the staff is important for good performance. It should involve all stakeholders with clear delineation of their duties for effective execution of mastitis control programme. Awareness camps to understand the responsibilities of various stakeholders and potential benefits for sustained involvement of the participants are also important.

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