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Model Training Course

on

Bovine mastitis: Theoretical and Practical Consideration in Management

1st to 8th March, 2013






Sponsored by

Directorate of Extension, Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India

Conducted at

Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS), Hebbal, Bangalore

Model Training Course

On

"Bovine mastitis: Theoretical and Practical Consideration in Management"

1st – 8th March, 2013

Training Manual

Compiled and Edited

by

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Sponsored by

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Conducted at

Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS), Hebbal, Bangalore – 560 024.

Acknowledgement

We put on record the encouragement by the Project Director, PD_ ADMAS, Bangalore for submitting the proposal for financial support. His mentorship from conceptualization to conduct of this training is gratefully acknowledged.

The financial help from Directorate of Extension, Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India for conducting the 8 days Model Training Course on "Bovine mastitis: Theoretical and Practical Consideration in Management" is gratefully acknowledged.

The logistic support provided by IVRI, NBAII, and NBLSSUP, Bangalore by providing accommodation to the participants is also gratefully acknowledged.

FOREWORD

India is the leading producer of milk in the world and accounts for 16.2% of the world milk production. Animal health is the backbone of the rapidly growing livestock industry. Dairy farming forms the second largest economic activity in India and over 90 per cent of milch animals are reared in unorganized sector by the small farmers and mastitis is a devastating disease affecting their livelihood. Bovine mastitis is a major setback in the dairy sector which causes huge loss to the nation's economy. Subclinical mastitis is found more important in India (varying from 10-50% in cows and 5-20% in buffaloes) than clinical mastitis (1-10%). Despite of advancing research to understand the complexity of the disease and advocacy of therapeutic measures mastitis still remains a global problem.

On the occasion of Silver Jubilee of our Institute we attempt to put-forth efforts to disseminate knowledge and understanding on the complexity of the disease including different managemental aspects of controlling mastitis and recent advances in the therapeutic field through this training programme. The model training programme on "Bovine Mastitis-theoretical and practical considerations in management" was aptly conceptualized by the Scientists of the Directorate and sponsored by the Directorate of Extension, Ministry of Agriculture and Cooperation, government of India, New Delhi. I hope this training will help the trainees to keep abreast the latest scientific understanding of bovine mastitis and implement the necessary managemental practices to reduce the occurrence of bovine mastitis in their region.

I extend warm welcome to our participants and wish them a memorable stay in this garden city with enriched knowledge.

(H. Rahman)
Course Director & Project Director

Preface

Mastitis is a major infectious disease affecting dairy cattle in herds. Because of its economic impact and due to the animal welfare policy, the pathogenesis of this intra-mammary infection was studied extensively over the past 50 years. In India, the estimated loss due to mastitis is around Rs. 7165.51 crores per annum. Although a wide spectrum of bacterial species have been identified as causative agents of mastitis, only staphylococci, streptococci and coliforms are major pathogens of economic and epidemiological importance. With the development of molecular biology techniques, microbiologists have more choices for characterization of these mastitis causing micro-organisms, which is vital for an effective mastitis control program. Despite of considerable research effort, mastitis remains the most costly disease in dairy cattle. Hence continuous monitoring of mastitis and its careful management is essential for the well being of dairy herd.

PD_ADMAS has been focussing on research in bovine mastitis since 2008. During the course of the research, tried to understand partially the molecular details of host-microbe interaction specially in staphylococcal and Coliform mastitis and developed molecular diagnostic methods for detection of major mastitis pathogens. This has laid the foundation for further in-depth understanding of the early immune response and complexities associated with mammary epithelial cell damage contributed by both the agents and the host factors. The model training course on **"Bovine mastitis: Theoretical and Practical Consideration in Management"** proposed by PD_ADMAS was approved by the Directorate of Extension, Department of Agriculture and Cooperation, Ministry of Agriculture, GOI, Krishi Vistar Bhawan, New Delhi realising the importance. I hope that the training course will provide a platform for the scientific interaction and exchange of ideas between scientists, field veterinarian and dairy entrepreneurs with some innovative ideas for the diagnosis of bovine mastitis and also in designing strategies for effective treatment and control. I am sure, the training manual brought out during the training will be handy to enhance overall understanding in management of mastitis.

I look forward and wish that the experienced participants will interact and share their knowledge for the benefit of all.

(B.R. Shome)
Course Director

Contents

Training Schedule

"Bovine Mastitis-theoretical and practical considerations in management"

(01.03.2013 to 08.03.2013)

Date& Day	Time	Topic	Venue	Speaker
01.03.2013 Friday	9:30-10:30 am	Registration	PD_ADMAS	NA
	10:30-11:00 am	Pre-training evaluation	Conference Hall, PD_ADMAS	Dr. B.R. Shome Principal Scientist and Dr. P. Krishnamoorthy, Scientist, PD_ADMAS , Bangalore
	11:00-11:20 am	Tea break		
	11:20-12.00 noon	Trainees view on Bovine mastitis	Conference Hall, PD_ADMAS	NA
	12.00 -1.30 pm	Visit to labs in PD_ADMAS		
	1:30-2:30 pm	Lunch break		
	2:30-4:00 pm	General overview and etiology of Bovine Mastitis	Conference Hall, PD_ADMAS	Dr. B.R. Shome Principal Scientist, PD_ADMAS , Bangalore
	4:00- 5:00 pm	Inauguration	Conference Hall, PD_ADMAS	NA
02.03.2013 Saturday	9:30-11:00 am (theory)	Pathogenesis of Bovine Mastitis	Conference Hall, PD_ADMAS	Dr. P. Krishnamoorthy, Scientist, PD_ADMAS, Bangalore
	11:00- 11:20 am	Tea break		
	11:20-1.30 pm (theory)	Trends in diagnosis of Bovine Mastitis	Conference Hall, PD_ADMAS	Dr. Raveendra Hegde Scientist 3, IAH & VB
	1:30-2:30 pm	Lunch break		
	2:30- 5:00 pm (practical)	Demonstration of conventional and molecular methods of mastitis diagnosis	Mastitis lab in PD_ADMAS	Dr. B.R. Shome Principal Scientist and Dr. P. Krishnamoorthy, Scientist, PD_ADMAS, Bangalore
03.03.2013 Sunday	9.30 – 5.00pm	Field trip to organized herds and unorganized dairy sectors		Dr. B.R. Shome Principal Scientist and Dr. P. Krishnamoorthy, Scientist, PD_ADMAS , Bangalore
04.03.2013 Monday	9:30-11:00 am	Economic impacts of Bovine Mastitis	Conference Hall, PD_ADMAS	Dr. Ganesh Kumar Senior Scientist and Dr. Govindaraj, Scientist, PD_ADMAS, Bangalore
	11:00- 11:20-am	Tea break		
	11:20-1:30 pm	Selection of dairy cows with reference to udder conformation	Conference Hall, PD_ADMAS	Dr. R. Bhaskaran Prof. and Head, Dep. of Livestock production and management, KVAFSU, Bangalore

Date& Day	Time	Topic	Venue	Speaker
	1:30-2:30 pm	Lunch break		
	2:30- 5:00 pm (Practical)	Demonstration of different bovine udder conformation with respect to mastitis	Visit to two local Farms	Dr. R. Bhaskaran Professor, Dep. of Livestock production and management, KVAFSU, Bangalore
05.03.2013 Tuesday	9:30-11.00 am (theory)	Breed variation in mastitis resistance-Role of Genomics	Conference Hall, PD_ADMAS	Dr. D. N. Das Principal Scientist, NDRI, Bangalore
	11:00-11:20 am	Tea break		
	11.20-1:30 pm (theory)	Nutritional management of dairy cows to prevent mastitis	Conference Hall, PD_ADMAS	Dr. N. K. S. Gowda Principal Scientist, NIANP, Bangalore
	1:30-2.30 pm	Lunch break		
	2:30-5:00 pm (practical)	Demonstration of different types of milking and introduction to milking machine	NDRI Cattle Yard Bangalore	Dr. D. N. Das and Dr. Jeyakumar NDRI, Bangalore
06.03.2013 Wednesday	9:30-11:00 am (theory)	Dairy herd management practices for control of mastitis	Conference Hall, PD_ADMAS	Dr. D. N. Das Principal Scientist, NDRI, Bangalore
	11:00-11.20 am	Tea break		
	11:20-12:20 pm	Clean milk production with emphasis on HACCP	Conference Hall, PD_ADMAS	Dr. D. N. Das Principal Scientist, NDRI, Bangalore
	12:20-1:30 pm	Decision support system in mastitis management	Conference Hall, PD_ADMAS	Dr. Manjunatha Reddy, Scientist, PD_ADMAS, Bangalore
	1:30 pm-2:30 pm	Lunch break		
	2:30 – 5:00 pm (practical)	Visit to Bangalore Mega Dairy and chilling plant Karnataka Milk Federation		
07.03.2013 Thursday	9:30-11:00 am (theory)	Recent advances in Bovine mastitis treatment	Conference Hall, PD_ADMAS	Dr. P. Vijayalakshmi, Assistant professor, RAGACOVAS, Puducherry
	11:00-11:20 am	Tea break		
	11:20-1:30 pm (theory)	Clinical approaches in treatment of mastitis at field level.	Conference Hall, PD_ADMAS	Dr. H. A. Upendra, Prof. and Head Dept of Clinical Medicine, KVAFSU, Bangalore
	1:30-2:30 pm	Lunch break		

Date & Day	Time	Topic	Venue	Speaker
07.03.2013 Thursday	2.30-3.30 pm	Alternative therapy for Bovine Mastitis	Conference Hall, PD_ADMAS	Dr. V.N. Vishwanatha Reddy, Former Prof. & Head, Dept of Animal Reproduction, Gynaecology & Obstetrics, Veterinary College, Bangalore. Presently Consultant to KMF, Bangalore
	3:30 – 5:00 pm (practical)	Demonstration of ABST, MIC etc	Mastitis lab in PD_ADMAS	Dr. B. R. Shome and Dr. P. Krishnamoorthy, PD_ADMAS, Bangalore
08.03.2013 Friday	9:30-11:00 am (theory)	Application of nanotechnology with special reference to Bovine Mastitis.	Conference Hall, PD_ADMAS	Dr. B. M. Veere Gowda Assistant Professor Dept of Vet Microbiology KVAFSU, Bangalore.
	11:00-11:20 am	Tea break		
	11:20-12:30 pm (theory)	Research needs in Bovine mastitis	Conference Hall, PD_ADMAS	Dr. B.R. Shome Principal Scientist, PD_ ADMAS , Bangalore
	12:30-1:30	Post- training evaluation	Conference Hall, PD_ADMAS	Dr. B.R. Shome Principal Scientist and Dr. P. Krishnamoorthy, Scientist, PD_ADMAS
	1:30-2:30 pm	Lunch break		
	2:30-:4.30 pm	Valedictory function	Conference Hall, PD_ADMAS	Chairman, Project Director, PD_ADMAS, Bangalore

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An Overview on Bovine Mastitis – the major concern of Dairy Industry

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Introduction

Bovine mastitis (*mast* = breast; *itis* = inflammation), is a major disease affecting dairy cattle worldwide. It is an inflammatory disease of cow and buffaloes mammary gland caused by various infectious or non-infectious etiological agents. The occurrence of disease is an outcome of interplay between the infectious agents and management practices stressing the defence of udder. According to Kennedy and Miller (1993), mastitis is expressed by tissue injury caused by tissue invasive or toxigenic organisms, which become dominant due to upset of balance in microbial population.

Mastitis must have been one of the first observed diseases of farm animals when cattle were domesticated over 5000 years ago. Since, then it has been an ever existing problem for all those who kept and milked dairy cattle and buffaloes. Milk production alone involves more than 70 million producers in India, each raising one or two cows/ buffaloes primarily for their livelihood. Although, India is the largest milk producer in the world (Fig. 1), bovine mastitis remains one of the important production diseases of dairy animals which directly or indirectly affect the economy of the farmers and ultimately affect the economy of the country.

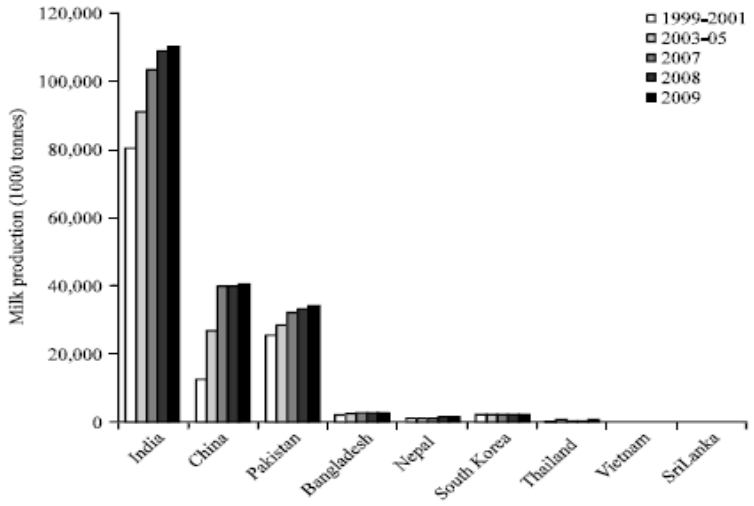


Fig. 1:The milk production trend in Asian countries

The severity of the inflammation can be classified into sub-clinical, clinical and chronic forms, and its degree is dependent on the nature of the causative pathogen and on the age, breed, immunological health and lactation state of the animal. Sub-clinical mastitis is difficult to detect due to the absence of any visible indications, and it has major cost implications. Chronic mastitis is a rare form of the disease but results in persistent inflammation of the mammary gland. This review gives a brief overview of the economic impact of bovine mastitis, its epidemiology and critically assesses recent advances in mastitis diagnostics.

Impact of Bovine Mastitis

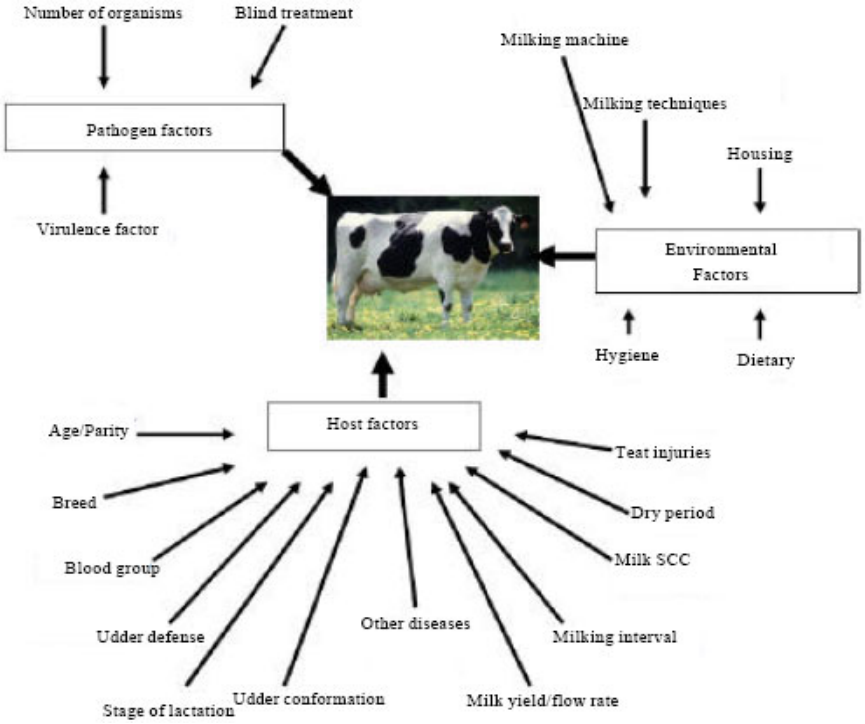
Mastitis is a global problem as it adversely affects animal health, quality of milk and economics of milk production and every country including developed ones suffer huge financial losses .India is the highest milk producer in the world but the per capita availability of milk still remains half of the world

average, demanding strategic intervention. One of the reasons for low productivity is poor animal health, particularly, mastitis which is single largest problem in dairy animal in terms of economic losses in India. It is proved by the reports that the annual economic losses due to bovine mastitis has increased 135 folds in about almost 5 decades from 1962 (INR 529 million/annum) to 2009 (INR 71655.1million/annum). The dramatic increase in the economic losses due to mastitis, divert the mind of researchers, policy makers and dairy farmers towards control of this costliest disease. In addition to heavy losses in milk quality and quantity, it also causes irreversible damage to the udder tissue and less occasional fatalities. Mastitis destroys the milk-secreting cells. Scar or connective tissue replaces the milk secreting tissue, resulting in a permanent loss of productive ability. Mastitis can lead to the reduction of offspring to a given production system due to the insufficient milk production resulting in starvation.

Risk factors of Mastitis

Risk factors such as management practices (shed and udder hygiene, poor teat condition, poor environmental hygiene, sanitation, large herd size, use of hand wash cloth, improper teat dipping), host (breed, high yielder, udder immunity, teat lesions, genetic resistance) and diet (Cu, Co, Zn, Selenium and vitamin E deficiency) amongst others have been reported to be important in the prevalence and epidemiology of both clinical and sub-clinical mastitis. To simplify understanding of the mastitis complexity, it is useful to consider risk factors or disease determinants which are broadly classified into three groups- host (cow or buffalo), pathogen (micro-organisms) and environment (Fig. 2).

Fig. 2: Risk factors for bovine mastitis



Etiology

Mastitis is a multi-etiological complex disease. Most major pathogens were identified by the 1940s. More than 250 infectious causes of bovine mastitis are known to date and in large animals the most common pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae*, other *Streptococcus* and Coliforms in Asia. CNS is a recently emerging pathogen causing bovine mastitis. The predominance of a bacterial species may vary according to the geographical region under scrutiny. *S. aureus* is one of the significant pathogens causing mastitis in dairy ruminants in many countries.

Table 1: Common mastitis causing microorganisms in the world including Asian countries

Bacteria	*Staphylococcus aureus, Streptococcus agalactiae, Escherichia coli, Streptococcus dysgalaciae, Streptococcus uberis, Streptococcus zooepidermidis, Streptococcus faecalis, Streptococcus pyogenes, Corynebacterium pyogenes, Pseudomonas aeruginosa, Mycoplasma bovis, M. Canadensis, M. californicum, M. bovihirnis, M. dispar, M. bovigenitalium, M. alkalescens, Campylobacter jejuni, Haemophilus somnus, Streptococcus pneumoniae, Corynebacterium ulcerans, Klebsiella pneumoniae, K. oxytocia, Enterobacter aerogenes, Mycobacterium bovis, M. tuberculosis, M. lacticola, M. fortuitum, Bacillus cereus, Pasteurella multocida, P. Haemolytica, Bacteroides funduliformis, Serratia marceseens, Acholeplasma laidlawii, Yersenia pseudotuberculosis, Mannheimia haemolytica, Mannheimia granulomatis.*
Facultative bacteria	*Peptococcus indolicus, Bacteroides melaninogenius, Eubacterium combesii, Clostridium sporogene, C. perfringens* type A and *Fusobacterium necrophorum, Citrobacter, Proteus*
Fungi / Yeast	*Aspergillus fumigatus, A. nidulans, Trichosporon cutaneum, Trichosporon beigelii, Pichia spp., Geotrichum candidum, Nocardia asteroids, N. brasiliensis, N. farcinica, N. neocaledoniensis, Candida krusei, C. tropicalis, C. paratropicalis, C. quillermondii, C. rugosa, Cryptococcus neoformans, Saccharomyces spp., Torulopsis* etc.
Algae	*Prototheca zopfii, P. trispora, P. wickerhamii, P. blaschkeae* etc.
Viruses	Adeno virus, Herpes virus, Rota virus, Reo virus, Mammilitis virus, Pseudocowpox virus, Parainfluenza virus, Apthovirus, R.P. virus, Bovine immunodeficiency virus etc.

Generally, the mastitis due to fungi and yeast is uncommon or rare. But a low prevalence of fungal mastitis of 2 to 7% has been reported. The prevalence of mycotic mastitis is usually very low (1-12% of all mastitis causes) but sometimes it can occur in enzootic proportions.

Epidemiology of Bovine Mastitis

The prevalence of mastitis is increasing in parallel with the development of new high milk producing breeds of cows and buffaloes. Studies conducted in different states of India reflect the high prevalence of bovine mastitis all over India for the past seven decades when the first record of the mastitis was made by Land in 1926. This significant increase in the occurrence of bovine mastitis is an alarming phase for the dairy sector. The increasing trend of bovine mastitis prevalence in Asian countries is depicted in Fig.3.

It has been reported that sub-clinical mastitis is 3- 40 times more common than the clinical mastitis and causes the greatest overall losses in most dairy herds. Only sub-clinical mastitis is responsible for 60-70% of total economic losses associated with all mastitic infections as it frequently goes unnoticed.

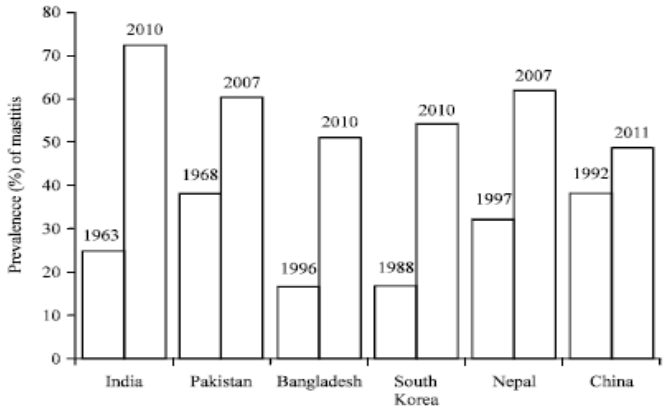


Fig. 3: The increasing trend of bovine mastitis prevalence

Current approaches for diagnosis of mastitis

Early diagnosis is of the utmost importance due to the high costs of mastitis. Diagnostic methods have been developed to check the quality of the milk through detection of mammary gland inflammation

and diagnosis of the infection and its causative pathogens. Currently, assays often used include measurement of SCCs, enzymatic analysis and the California milk clotting test. Colourimetric and fluorometric assays have been developed for measuring the concentrations of enzymes elevated in milk during mastitis (e.g. NAGase or LDH). Use of culturing techniques for the detection of mastitis-causing microorganisms is still the gold standard, although it is very labour-intensive and therefore expensive.

Recent Laboratory development in the detection of mastitis (Molecular Methods for Diagnostic Purpose)

As several bacterial species are involved in mastitis infection, inadequate pathogen detection or identification techniques often delay timely interventions in disease treatment and control. The early and accurate identification of pathogens will enable timely interventions for the treatment and control of bovine mastitis

Multiplex PCR

Recently an mPCR assay was developed which provides a convenient means of accurately identifying 10 important bacterial pathogens namely, *Staphylococcus aureus, Staphylococcus chromogenes, Staphylococcus epidermidis, Staphylococcus sciuri, Staphylococcus haemolyticus, Staphylococcus simulans, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis* and *Escherichia coli* in milk (Shome et al., 2011). This assay has made it possible to optimize reagent use, leading to lower assay costs. The assay will be of value in the dairy sector for diagnosis and research.

Real Time PCR

Among the first commercially available PCR based mastitis tests, developed for use with milk samples and including all reagents of DNA extraction and real time PCR, is the Patho Proof Mastitis PCR Assay (Finzymes Oy, Espo Finland). The assay is performed directly from raw or preserved milk and identifies a total of 11 mastitis causing bacterial species or species group and the b-lactamase gene (the bla Z gene) responsible for staphylococcal penicillin resistance.

Universal array for Pathogen Detection

In recent years, DNA microarray technology has played an increasingly important role in bacterial studies, including detection and quantitative analyses. More recently, described a 16S rRNA oligonucleotide microarray to identify 22 common pathogenic species, whereas developed a microarray for the detection of the main food borne pathogens. A microarray platform based o the discriminative properties of the DNA ligation detection reaction (LDR), associated with a universal tag array (UA) has been developed to identify the main pathogens known to cause mastitis in cattle, sheep and goats or those responsible for food borne intoxication or infection or both .

Biochip for rapid detection

Using microarray technology, a biochip capable of detecting and identifying a various strains of bovine mastitis pathogens (7 pathogens) within hours directly from milk have been developed. The complete biochip system could be suitable for clinical laboratories with expertise in molecular biology techniques despite the fact that the method requires relatively little hands on training. However because of the cost of the system lies in the DNA extraction of bacteria and multiplex PCR steps, the biochip system might not be affordable to farmers.

Pyrosequencing for milk microbiome signatures of subclinical mastitis

The on-going revolution in metagenomic sequencing technology has led to the production of sequencing machines with dramatically lower costs and higher throughput. Recently metagenomic analysis of milk samples collected from Kankrej, Gir (Bos indicus) and crossbred (Bos taurus × B. indicus) cattle harbouring subclinical mastitis was carried out by next-generation sequencing 454 GS-FLX technology to elucidate the microbial community structure of cattle milk. The analysis identified

potential infectious organisms in mastitis, resistance of organisms to antibiotics and chemical compounds and the natural resistance potential of dairy cows.

Mastitis Therapy

Effective and economical mastitis control programs rely on prevention rather than treatment. Targeting treatment towards specific pathogens when possible is considered necessary for rational antimicrobial treatment of mastitis, as should be the case in all treatment of bacterial infections. It is clear that mastitis caused by Gram-positive agents needs different approaches than mastitis caused by Gram-negative bacteria. Because new diagnostic tools such as PCR based tests have made rapid identification of mastitis-causing bacteria feasible, routine use of broad-spectrum antimicrobials without diagnosis could be considered as an outdated practice. Efficacy of supportive treatment such as non-steroidal anti-inflammatory agents, frequent milking and fluid therapy in mastitis has been studied, in combination with antibiotic treatment. Use of non-steroidal anti-inflammatory drugs has been shown to be beneficial at least in clinical mastitis. Herbal or homeopathic remedies though used alternatively, have not been shown to be effective for mastitis treatment in several independent, scientific studies.

Conclusions

Advances in mastitis research in the last decade or so have brought exciting new knowledge and technologies that can/will be used to solve complex problems confronting dairy production. A better understanding of the multiplicity of pathogens capable of causing mastitis, and knowledge of mammary gland immunology, bacterial virulence factors, and mechanisms of pathogenesis will definitely facilitate development of effective mastitis vaccines.

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Etiology of Bovine Mastitis

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Bovine Mastitis has been and continues to be recognized as one of the major disease affecting the dairy industry. Mastitis defined as 'inflammation of mammary gland' can have an infectious and non-infectious aetiology. Inflammation can be caused by many types of injury including infectious agents and their toxins, physical trauma or chemical irritants. Previously, the mastitis researchers associated the mastitis with the physical factors like cold and mechanical injuries only. It was Frank in the year 1876 who proved the infectious nature of this disease and put forward an entirely new concept in the investigation of bovine mastitis. An association between mastitis and pathogenic micro-organism was established in 1887. Although bacteria, fungi, yeasts and possibly virus can cause udder infections, the main agents are bacteria. Most major pathogens were identified by the 1940s. The occurrence of disease is an outcome of interplay between the infectious agents and management practices stressing the defense of udder. According to Kennedy and Miller (1993), mastitis is expressed by tissue injury caused by tissue invasive or toxigenic organisms, which become dominant due to upset of balance in microbial population. The recent scientific literature on mastitis is so vast that the people have concentrated on individual species of major mastitis pathogens and its various aspects of treatment and control.

Mastitis can be caused by over 250 different contagious and environmental microorganisms such as Gram-positive cocci, Gram-negative cocci (Coliforms especifically *E. coli*, *Enterobacter*, *Klebsiella* spp.) and other miscellaneous organisms, which include *Nocardia*, *Prototheca* and *Yeast*. A large number of bacteria have been reported to cause mastitis such as *Brucella melitensis*, *Corynebacterium bovis*, *Enterobacter aerogenes*, *Escherichia coli, (E. coli), Klebsiella oxytoca*, *Klebsiella neumoniae*, *Mycoplasma* (various species), *Pasteurella spp.*, *Proteus spp.*, *Prototheca wickerhamii* (achlorophyllic algae), *Prototheca zopfii* (achlorophyllic algae), *Pseudomonas aeruginosa*, *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Streptococcus agalactiae* (*S. agalactiae*), *Streptococcus uberis* (*S. uberis*), *Streptococcus dysgalactiae* (*S. dysgalactiae*), *Trueperella pyogenes* (previously *Arcanobacterium pyogenes*).

Of these, the most common mastitis causing pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis* and *Escherichia coli* though other pathogens can cause occasional herd outbreaks. Recently, a new species of bacteria (*Mannheimia granulomatis*) had been isolated from milk of subclinical bovine mastitis in Isreal.

The microorganisms causing mastitis can be grouped into three catagories: 1) Contagious (*S. aureus*, *S. agalactiae*, *C. bovis*, *Mycoplasma* spp.), 2) Environmental (*E. coli*, *K. pneumonia*, *K. oxytoca*, *Serratia* spp., *Citrobacter* spp., *S. uberis*, *S. bovis* and *S. dysgalactiae*) and 3) Other Coagulase negative staphylococci (CNS), *Pseudomona aeruginosa*, *Nocardia asteroids*, *Prototheca* spp., *Candida* spp.).

The prevalence of these mastitis pathogens varies between the countries (Table 1). However, *S. aureus* has been reported as the chief etiological agent of mastitis in Asian countries by various researchers. *S. aureus* is ubiquitous organism and can lead to peracute, acute, subacute, chronic, gangrenous and sub-clinical types of mastitis. Classically, CNS was classified as minor pathogens and their importance as an independent cause of subclinical or clinical mastitis was judged to be limited. However, the significance of CNS needs to be reconsidered as in many countries they have become the most common mastitis causing agents .In India, the prevalence of CNS in milk samples increased from 9.91% in 2003 to 72.13% in 2009. Thus, CNS are now emerging as a major pathogen associated with subclinical mastitis in many countries.

Table 1: Distribution of common organisms in different countries of Asia

	Prevalence (%) of common mastitis pathogens					
Country	*S. aureus*	CNS	*Strep.* Sp.	*E. coli*	Others	Reference
India	74.71	-	21.13	-	4.15	Singh *et al.* (1982)
	42.10	-	5.26	18.95	3.16[1], 4.74[2], 5.26[3], 2.10[7]	Misra *et al.* (1993)
	60.32	-	31.98	-	-	Shukla *et al.* (1998)
	74.04[#]	-	6.00	-	7.32[4], 2.93[5], 5.71[3],	Patel *et al.* (2000)
	39.01, 52.48*	-	-	-	8.51[6]	Ghose and Sharda (2003)
	18.99[#]	-	15.50	-	17.05[9]	Jha *et al.* (2004)
	27.90,	16.28	6.98[a]	17.44	5.81[1], 4.65[11], 5.81[12], 4.56[4], 3.49[2], 3.49[13], 3.49[7]	Das and Joseph (2005)
	-	-	15.45	12.73	10[5]	Sharma *et al.* (2007)
	34.38	25	9.38	21.87	6.25[2], 3.12[3]	Yathiraj *et al.* (2007)
	39.53[#]	-	20.93	9.30	16.27[6], 6.97[1], 6.97[2]	Vishwakarma (2008)
	16.66	40.47	33.33		9.52[6]	Ahire *et al.* (2008)
	59.37, 4.90*		16[a], 10.63[c], 1.1[b]	2.9	2.2[1], 1.8[4], 1.1[11], 1.1[12], 1.1[2]	Sahoo *et al.* (2009)
	27.86	72.13	-	-	-	Dutta (2009)
	27.27, 15.91*	-	50[b], 4.55[a]	-	2.27[12]	Kumar *et al.* (2009)
	57.27[#]	-	15.45	12.73	10[5]	Sharma and Maiti (2010)
	27.37	12.63	5.79	8.95	7.89[2], 1.35[14]	Ranjan et al. (2011)
	33.83	97.80	-	-	-	Krithiga *et al.* (2011)
Pakistan	12.06	-	7[d], 3[a]	10	3.5[5], 3[4]	Javed and Siddique (1999)
	33.99	-	-	27.09	35.46[13], 1.97[2], 1.48[5]	Rashid (2001)
	13.42[#]	-	9.39	46.98	14.77[6], 4.02[3], 2.01[15], 1.34[16]	Iqbal *et al.* (2004)
	45	-	23[a]	18	14[4]	Khan and Mohammad (2005)
	49.53, 6.54*	0.93	23.83[a], 8.88[c], 0.93[b]	1.4	3.74[4], 0.93[5], 0.47[14], 0.47[17]	Ali *et al.* (2008)
	28.32[#]	-	7.51	16.18	13.29[6], 12.42[4], 7.22[16], 6.64[5], 5.20[1], 3.17[18]	Ali *et al.* (2011)
South Korea	51.40, 2.7*	-	15.8[b], 2.1[a], 1[d]	16.50[ab]	5.1[4], 0.3[2], 0.3[15]	Kang-Hee *et al.* (2001)
	27.40	25.04	-	-	-	Moon *et al.* (2007)
	12.2	40.70	5.3[d],	4.5, 19.5[ab]	-	Nam *et al.* (2010)
Bangladesh	49.30[#]		14	6	8[5], 4.7[4],	Mahbub-E-Elahi *et al.* (1996)
	39.64[#]		2.47	11.11	3.7[4],	Kader *et al.* (2002)
	31	-	3.1	11.3	4.7[4]	Rahman *et al.*, 2010
China	23.81[#]	-	39.36	-	25.39[9]	Zhongwen *et al.* (2002)
	78.98	-	-	-	-	Liu *et al.* (2005)
	79.12, 6.59[$], 1.1[*]	-	2.2[b]	-	1.1[4], 1.1[1]	Liu *et al.* (2006)
	22.61[#]	-	25.22	-	15.65[13], 16.52[14], 6.96[5]	Wang and Niu (2009)
	41	-	53[d], 29[b], 27[a]	82	-	Cheng *et al.* (2010)
	29.5	4.7-19.7	7.6[a], 4.7[d], 2.9[b]	25.70	16.2[6]	Yang *et al.* (2011)
Iran	2.89	-	22.11[a], 11.43[b],	10.16	1.07[6], 1.76[4], 0.14[1], 0.21[2], 0.03[15], 0.03[19]	Atyabi *et al.* (2002)

Courtesy: Sharma et al., 2012

In some countries, mastitis due to the environmental pathogens poses major problems to the dairy industry. Environmental mastitis has become a major concern in many well-managed dairy farms that have successfully controlled contagious pathogens. In U.K, the contagious pathogens (*S. aureus, S. dysgalactiae*, *S. agalactiae*) accounted for only 10% of clinical cases. The main organisms associated with clinical mastitis in England and Wales are *E. coli* and *S. uberis*.

Mastitis caused by *Pseudomonas aeruginosa* rarely develops in cattle, and only as sporadic form following intramammary infusions of contaminated material. Mastitis caused by *Mannheimia haemolytica* and *Pasteurella spp.* are more common in sheep, but is rarely reported in cattle and is usually sporadic. Other mastitis that evolves sporadically in a herd, affecting one or two cows, can be caused by *Nocardia spp*., *Serratia* spp., *Mycobacterium bovis*, which is the etiologic agent of bovine tuberculosis, and other mycobacteria (*M. lacticola, M. fortuitum, M. phlei, M. smegmatis, M. chelonei* and *M. tuberculosis*). *Bacillus cereus* and *Bacillus subtilis* are saprophytic organisms, occasionally causing acute hemorrhagic mastitis in cattle. Mastitis caused by *Listeria monocytogenes* are important because of the zoonotic risk due to the consumption of contaminated dairy products. Also, mastitis rarely caused by *Clostridium perfringens* type A, has zoonotic risk and can cause food poisoning in humans.

Generally, mastitis due to fungi and yeast is uncommon or rare. A survey conducted in different countries on rate of isolation of fungi from milk revealed isolation rate of 6.1% in Egypt, 1.3% in South Korea and 25.4% in Brazil. Among Fungi, *Candida* spp., *Aspergillus* spp, *Trichosporon* spp. and *Saccharomyces* spp. are more prevalent. The frequency of isolation of potentially pathogenic yeast from milk samples has been shown to be about 7% in central and northern Europe and also in the USA. Among the algae, *Prototheca zopfii, Prototheca wickerhamii, Prototheca trispora and Prototheca blaschkeae* have been reported to cause bovine mastitis. In India, however, bacteria are the major causative agent of mastitis and prevalence of mycotic mastitis is very less.

Mastitis still remains a complex disease condition difficult to resolve due to the involvement of such a wide range of etiological agents. Considering that different pathogens are the predominant cause of mastitis in different countries, mastitis controls will need to be developed to meet the specific requirements of an individual country or segment of the dairy industry. Recent scientific developments, specifically in the areas of genomics and proteomics at host and pathogen level, provide exponentially growing opportunities for deepening our understanding of mastitis pathogenesis and epidemiology which will pave way for devising effective control strategies.

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Pathogenesis of Bovine Mastitis

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Bovine mastitis (mast = breast; itis = inflammation), a major disease affecting dairy cattle worldwide, results from the inflammation of the mammary gland. The severity of the inflammation can be classified into sub-clinical, clinical and chronic forms, and its degree is dependent on the nature of the causative pathogen and on the age, breed, immunological health and lactation state of the animal. Sub-clinical mastitis is difficult to detect due to the absence of any visible indications, and it has major cost implications. Chronic mastitis is a rare form of the disease but results in persistent inflammation of the mammary gland. The direct effects of mastitis are temporary or permanent loss in milk production, poor milk quality, reduction in price, treatment costs, labour costs, premature culling, etc.

The primary cause of mastitis is a wide spectrum of bacterial strains. The teat end serves as the first line of defense against infection. Normally, the teat canal is tightly closed by sphincter muscles, preventing the entry of pathogens and also escaping of milk. It is lined with keratin, a waxy material derived from stratified squamous epithelium that obstructs the migration of bacteria and contains antimicrobial agents, such as long-chain fatty acids, that assist in combating the infection. However, the efficiency of keratin is restricted. The fatty acids are both esterified and non- esterified, representing myristic acid, palmitoleic acid and linolenic acid which are bacteriostatic. The fibrous proteins of keratin in the teat canal bind electrostatically to mastitis pathogens, which alter the bacterial cell wall, rendering it more susceptible to osmotic pressure. Inability to maintain osmotic pressure causes lysis and death of invading pathogens. The keratin structure thus enables trapping of invading bacteria and prevents their migration in to the gland cistern. Damage to keratin has been reported to cause increased susceptibility of teat canal to bacterial invasion and colonization (Bramley and Dodd, 1984). Fluid accumulates within the mammary gland as parturition approaches, resulting in increased intramammary pressure and mammary gland vulnerability caused by the dilation of the teat canal and leakage of mammary secretions. Additionally, during milking, the keratin is flushed out and there is distention of the teat canal. The bacteria present near the opening of the teat find opportunity to enter the teat canal, causing trauma and damage to the keratin or mucous membranes lining the teat sinus. The sphincter requires ~2 hours returning back to the contracted position. Bacterial pathogen which are able to traverse the opening of teat end by escaping antibacterial activities establish the disease process in the mammary gland which is the second line of the defense of the host. Once inside the teat, bacteria must also elude the cellular and humoral defense mechanisms of the udder. In dairy animals, the mammary gland has a simple system consisting of teats and udder, where the bacteria multiply and produce toxins, enzymes and cell wall components which stimulate the production of inflammatory mediators attracting phagocytes. They liberate toxins and induce leukocytes and epithelial cells to release chemo attractants, including cytokines such as tumour necrosis factor-α (TNFα), interleukin (IL)-8, IL-1, eicosanoids (like prostaglandin F2α [PGF2α]), oxygen radicals and acute phase proteins (APPs) (e.g. haptoglobin [Hp], serum amyloid A [SAA]). This attracts circulating immune effector cells, mainly polymorphonuclear neutrophils (PMNs), to the site of infection. The severity of inflammatory response however is dependent upon both the host and pathogen factors. The pathogen factors include the species, virulence, strain and the size of inoculums of bacteria; where as the host factors include parity, the stage of lactation, age and immune status of the animal, as well as the somatic cell count.

PMNs act by engulfing and destroying the invading bacteria via oxygen-dependent and oxygen-independent systems. They contain intracellular granules that store bactericidal peptides, proteins, enzymes (such as myeloperoxidase) and neutral and acidic proteases (such as elastase, cathepsin G,

cathepsin B and cathepsin D). The released oxidants and proteases destroy the bacteria and some of the epithelial cells, resulting in decreased milk production and release of enzymes, such as N-acetyl-β -D-glucosaminidase (NAGase) and lactate dehydrogenase (LDH). Destruction of most of the PMNs takes place by apoptosis once their task is fulfilled. Subsequently, macrophages engulf and ingest the remaining PMNs. The dead and sloughed off mammary epithelial cells, in addition to the dead leukocytes, are secreted into the milk, resulting in high milk SCCs. If the infection persists, internal swelling within the mammary epithelium, not normally detectable by an external examination, can occur. The mammary gland alveoli become damaged and start losing anatomical integrity. The blood–milk barrier is breached, causing extracellular fluid components, such as chloride, sodium, hydrogen, potassium and hydroxide ions, to enter the gland and mix with the milk. When extensive damage to the blood–milk barrier has occurred, blood might be detected in the milk. This leads to visible changes on the udder, such as enhanced external swelling and reddening of the gland. Changes also occur in the milk, including increased conductivity, increased pH, raised water content and the presence of visible clots and flakes. This marks the initial stage of clinical symptoms, and the most severe infections might ultimately result in the death of the animal (Viguier *et al*., 2009).

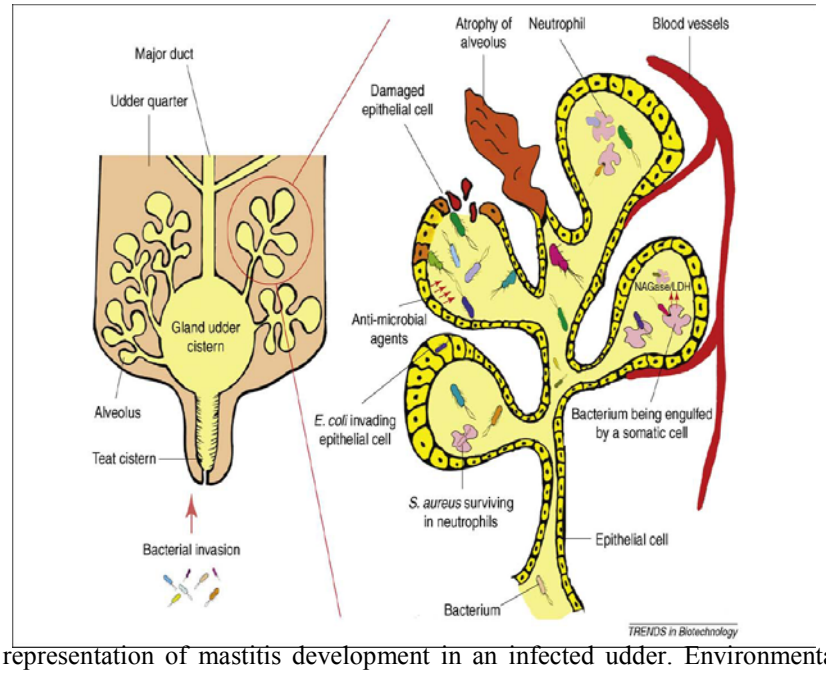

Figure 1. Schematic representation of mastitis development in an infected udder. Environmental and contagious microorganisms invade the udder through the teat cistern. They then multiply in the udder where they are attacked by neutrophils while damaging the epithelial cells lining the alveoli, with subsequent release of enzymes like NAGase and LDH. The epithelial cells also secrete anti-microbial compounds. Considerable tissue damage is observed once the immune effector cells begin to combat the invading pathogens.

Macrophages are the predominant cells found in milk and tissue of healthy involuted and lactating mammary glands. It ingests bacteria, cellular debris and accumulated milk components. The phagocytic activity of macrophages can be increased in the presence of opsonic antibody for specific pathogens. Because of indiscriminate ingestion of fat, casein and milk components, the mammary gland macrophages are less effective at phagocytosis than are blood leukocytes. Macrophages play a role in antigen processing and presentation. Conditions which contribute to trauma of mammary gland include: incorrect use of udder washes, wet teats and failure to use teat dips, failure to prepare milking animals or premilking stimulation for milk ejection, over milking, insertion of mastitis tubes or teat canulae, injury caused by infectious agents and their toxins and physical trauma (Khan and Khan, 2006). Jones (1998) described the pathogenesis of *S. aureus* mastitis, which produced toxins that destroy cell membranes and could directly damage milk producing tissue. Initially, the bacterium damages the tissue lining the teat

and gland cisterns within the quarter. Then they move up into the duct system and establish deep seated pockets of infection in the alveoli. This was followed by walling of bacteria by scar tissue and formation of abscesses which might cause poor response to antibiotic treatment. Alvelolar and duct cells were destroyed and there was reduction in milk yield. These degenerated cells may combine with leukocytes and clog the milk ducts that drain the alveolar areas contributing for scar tissue formation. The abscess becomes quite large and detected as lumps in the udder swelling and chunks of milk clinically.

S. epidermidis, S. hyicus, S. xylosus were able to adhere and internalize bovine mammary cells in a process that appear to be receptor mediated and exploited host signal transduction and cytoskeleton to induce an uptake signal. The *S. xylosus* showed highest adherence and internalization values of the species evaluated (Almeida and Oliver, 2001). Mastitis results once bacteria pass through the teat duct and multiply in milk-producing tissues. Microorganisms breach the teat duct in several ways. Between milkings, microorganisms may pass through the teat duct by multiplying inside the duct or by physical movement resulting from pressure placed on the teat end as the cow moves about. During machine milking, microorganisms may be propelled into or through the teat duct into the teat cistern. The potential for invasion is greatly increased by bacteria that reside in or colonize the teat duct. Such colonizations occur in both lactating and dry cows and the colonizing bacteria may survive for months, serving as sources of bacteria for infecting the gland.

To study the pathogenesis of various microorganisms causing bovine mastitis by using bovines as experimental animals or laboratory animal models like mice, rat and rabbits. Chandler (1970) reported the first successful induction of experimental bacterial mastitis in mice using *Staphylococcus aureus, Streptococcus agalactiae, Corynebacterium pyogenes, Escherichia coli* and *Pseudomonas aeruginosa* isolated from cases of bovine mastitis. Brouillette *et al*. (2004) reported that mouse model of infectious mastitis was suitable for the primary evaluation of intramammary infection with a pathogen such as *Staphylococcus aureus* in both intracellular and extracellular phases of growth. Brouillette and Malouin (2005) reported that the mouse was a valuable model to study *S. aureus* mastitis in spite of differences in the mammary gland of bovines and mouse. Notebaert and Meyer, (2006) indicated that mouse mastitis model was straight forward and suitable model for the study of bovine mastitis. They also suggested that this was a valuable tool which provides information about pathogenic mechanisms of variety of organisms in the context of intramammary infections. Shpigel *et al*. (2008) suggested that the mouse model was a powerful tool in dissecting the molecular and genetic basis for the infection process in *E. coli* induced experimental mastitis. Amorena *et al*. (1991) developed an experimental model in rabbit to study the pathogenesis of mastitis.

Thus the studies of pathogenesis of various microorganisms are important to understand basic host pathogen interaction mechanisms by using various animal models. It will pave the way for better treatment and control strategies for bovine mastitis.

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Trends in Diagnosis of bovine mastitis

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Introduction

Bovine mastitis, defined as an inflammation of the mammary gland is a major disease affecting dairy cattle worldwide. It remains a major global challenge to milk production even in the face of wide spread implementation of mastitis control strategies. Despite the significant advances in understanding the disease, both clinical and sub-clinical mastitis remain a problem in dairy herds and prevalence rates in many countries remain similar to those observed decades ago. The prevalence of bovine mastitis continues to affect the dairy herds throughout the world in spite of continued research activity on the problem over the century. Mastitis negatively affects the quality of milk, milk production, farm economics and animal welfare. It is estimated that mastitis alone accounts to about 70 per cent of all avoidable losses incurred during milk production. In India, the overall economic loss due to mastitis is estimated to be Rs.7165.51 crores, a number which increased from 1607.2 crores a decade and half before. Subclinical mastitis is considered economically the most important in modern dairy herds, affecting 20% to 50% of cows in given herds Although the loss due to subclinical mastitis (SCM) is difficult to quantify, most experts agree that it costs the average dairy farmer more than the clinical mastitis does.

The disease usually occurs primarily in response to intramammary bacterial infection, but also to intramammary mycoplasmal, fungal, or algal infections. The severity of the inflammation can be classified into sub-clinical, clinical and chronic forms, and its degree is dependent on the nature of the causative pathogen and on the age, breed, immunological health and lactation state of the animal. Sub-clinical mastitis is difficult to detect due to the absence of any visible indications, and it has major cost implications. Chronic mastitis is a rarer form of the disease but results in persistent inflammation of the mammary gland.

Approaches for diagnosis of mastitis

Milk selected for human consumption must originate from healthy animals; hence early diagnosis is of utmost important. The diagnostic methods have been developed to check the quality of the milk through detection of mammary gland inflammation and diagnosis of the infection and causative pathogens. The traditional diagnostic methods described below are the most common ways of diagnosing bovine mastitis at the farm level worldwide. They are known to be used widely in detecting bovine mastitis. Despite of several major drawbacks in these methods, they still are widely used in diagnosing bovine mastitis in dairy herds.

Somatic Cell Count (SCC)

Somatic cell count is the most frequently used indicator of subclinical mastitis in dairy cattle. Automated devices have been developed recently so that rapid determination of somatic cell counts can be performed by using the Coulter Milk Cell Counter; where the particles are counted through an electric field and the Fossomatic; where the cells are stained using fluorescent dyes before counting the fluorescent particles.

California mastitis test (CMT): This assay indirectly measures the SCC in milk samples. A bromocresol-purple-containing detergent is used to break down the cell membrane of somatic cells, and the subsequent release and aggregation of nucleic acid forms a gel-like matrix with a viscosity that is proportional to the leukocyte number.

Electrical conductivity (EC) test: This test measures the increase in conductance in milk caused by the elevation in levels of ions such as sodium, potassium, calcium, magnesium and chloride during inflammation.

pH test: The rise in milk pH, due to mastitis, is detected using bromothymol blue.

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Current approaches for diagnosis of mastitis

Currently, measurement of SCCs, enzymatic analysis and California milk clotting test assays are oftenly used. Colourimetric and fluorometric assays have been developed for measuring the concentrations of enzymes elevated in milk during mastitis (e.g. NAGase or LDH). Use of culturing techniques for the detection of mastitis-causing microorganisms is still the gold standard, although it is very labour-intensive and therefore expensive.

Mastitis can also be detected using 'cow-side' or 'on-site' tests, which can be used by both farmers and veterinarians and which require relatively little training. California mastitis test (CMT) is one of the oldest and best known test and is based on the principle that the addition of a detergent to a milk sample with a high cell count will lyse the cells, release nucleic acids and other constituents and lead to the formation of a 'gel-like' matrix consistency. Mastitis can also be detected via changes in conductivity or pH. Although these effects are easy to monitor, they are relatively insensitive. Thus, there is a major need for new biomarkers that are specific for mastitis, easy to detect, occur at a very early stage and that can be measured 'on-site'.

Development of new biomarkers for mastitis:

Advances in relevant proteomics techniques, such as two-dimensional gel electrophoresis (2D-GE) and mass spectroscopy (MS) have led to the identification of several new proteins involved in mastitis. The proteomics studies resulted in information on the different protein expression pattern obtained from mastitis-infected milk and on the proteins expressed by invading pathogens example: six chaperonins with a role in pathogen recognition only in mastitic samples have been identified and therefore have potential as new markers for mastitis. Proteomic analysis of bovine neutrophils has resulted in the identification of over 250 proteins, of which 19 are known to be involved in the immune response of the host. They could potentially be used as markers for its detection, as reduced neutrophil function has been correlated with mastitis. This information can be applied not only to the discovery of new therapeutic targets but also to the search for new diagnostic bio-markers. However, the successful application of these new biomarkers in a detection device still remains a challenge.

Immunoassays, such as enzyme-linked immunosorbent assay (ELISA) can provide a reliable and inexpensive approach provided that suitable antibodies are available against specific inflammation-related biomarkers or the causative microorganisms. There have also been significant developments in nucleic-acid-based testing for the identification of the causative microorganisms. Immunoassays can also be used to detect inflammation-related biomarkers present in the milk at different stages of sub-clinical mastitis. For example, heptoglobin(Hp) concentrations have been reported to increase significantly in plasma, as well as in milk, during mastitis and thus Hp was suggested as a potential marker for diagnosis

The application of biomarker-based assays, developed within the last decade, has already shown considerable promise for mastitis detection. Nevertheless, additional studies on the validation of these assays for mastitis detection are required.

Nucleic acid testing:

At present, the genome sequences of many of the major mastitis-causing pathogens are available and hence can be utilized to develop nucleic acid-based testing methods, such as PCR. Such tests though are generally more expensive (than, for example, immunoassays) but they are highly sensitive and specific, can be performed rapidly (e.g. 'real-time' PCR) and can overcome the sensitivity and time-constraints sometimes encountered with culture-based tests and thus could complement or replace them in the long-term.

PCRs allow the identification of closely related organisms within a few hours. Multiplex PCR and 'real-time' PCR assays that can simultaneously detect different mastitis-causing organisms in milk samples have been described and the most recently developed assay is capable of detecting 11 of the major mastitis-associated pathogens, including *E. coli, S. aureus, Streptococcus agalactiae* and *Streptococcus uberis*.

Recent developments in 'cow-side' tests for the detection of mastitis

Rapid, 'cow-side' mastitis tests could be used by farmers and veterinarians to diagnose and treat the inflammation in its early stages, thus having the potential to stop the propagation of the disease in the herd. An increase in temperature is one of the symptoms associated with mastitis. A thermal camera was used to diagnose experimentally-induced mastitis and could detect temperature changes of 1 to 1.58^0C. Infrared thermography (IRT) has been employed as a diagnostic method in bovine mastitis based on heat detection generated from the udder. The thermal camera used in this method absorbs infrared radiation and based on the amount of heat generated, the images are produced and generated. Infrared thermography was also used to measure skin surface temperatures in infected cows and a strong correlation (R2 = 0.92) between skin surface temperature and SCCs was observed. This non-invasive approach can be used 'on-site.' Estimation of the levels of inflammation-related enzymes might also be used for the detection of mastitis as these show good correlation with SCCs. For example, an LDH activity assay was carried out using dry chemistry and a portable spectrophotometer with comparable variation coefficients to the assays performed in a laboratory environment. Other enzymatic tests include the detection of an esterase secreted by somatic cells using an enzymatic assay. Bioluminescence-determination assays based on estimation of the ATP concentrations in somatic cells or the recognition of somatic cell DNA by fluorescent staining, can also be used 'on-site' for the reliable determination of elevated SCC levels and thus the probable presence of mastitis.

Current and new trends in mastitis detection during automatic milking: Robotic milking has increased significantly over the past 15 years. It provides an ideal format for 'on-line' mastitis monitoring and 4 per cent of Dutch farmers now use this method.

Any 'on-line' mastitis detection is currently performed using electrical conductivity (EC), SCCs or colour determination, with milk EC being the most commonly used 'on-line' test. However, although a change in conductivity might be a useful indicator, on its own it is not a reliable or sensitive parameter for conclusive diagnosis. Milk colour analysis has also been used in automatic milking systems for the indication of mastitic infection, as the presence of a yellow colour or of blood in the milk, might be highly indicative of mastitis. However, the milk fat content can also influence colour, and some colour sensors failed to detect sub-clinical mastitis. Therefore, the development of novel sensors with higher sensitivities is the goal of many recent research efforts.

A chemical-array-based sensor, termed an 'electronic tongue', was able to detect chloride, potassium and sodium ions released during mastitis in addition to inorganic and organic cations and anions. This sensor could successfully discriminate between normal and mastitic milk samples with a specificity and sensitivity of 96% and 93%, respectively. Milk from mastitic and healthy cows could be distinguished using a gas-sensor-array system, or 'electronic nose'. It consisted of several gas sensors that interact with volatile substances, in-cluding sulphides, ketones, amines and acids. More recently, it was able to identify different pathogens, such as *S. aureus*, coagulase-negative staphylococci, *streptococc*i and *E. coli*, and to determine infection-free udder quarters based on the detection of the patterns of volatile metabolites produced. Elevated levels of lactate can also be used for the detection of early stages of mastitis. Limitation of oxygen availability in the mammary glands will lead to increases in the levels of lactate that are directly proportional to the level of metabolic activity. Lactate concentrations detected during mastitis infection showed positive correlation with SCCs

Biosensors have also been developed to detect mastitis. In this a biological receptor molecule (e.g. antibody, enzyme, nucleic acid) is used in combination with a transducer to produce an associated signal, allowing observation of a specific biological event (e.g. an antibody-antigen interaction).

Recent advances in microfluidics and so-called 'biochips' (which are also referred to as a 'laboratory-on-a-chip') have the capacity to revolutionize diagnostics, and these technologies have already been applied for the detection of mastitis. The disposable microchips to be used with a portable reader system to measure milk SCC. The milk sample is mixed with a lysis solution to burst the somatic cells, and a fluorescent dye is added to stain the DNA. The sample is then applied to the microchip, which uses a capillary flow to allow even distribution of the sample, and the fluorescence is measured with the portable reader system. Recently a biochip that incorporated DNA amplification of genes that are specific for seven known mastitis-causing pathogens has been developed. The incorporation of micro fluidics-based technologies into chip design has made it possible to significantly reduce reagent volumes, leading to lower assay costs and faster results, and also to determine several targets on one platform, which can improve assay efficiency, specificity and sensitivity and thus ultimately might lead to better mastitis treatment. In theory, all these assays could be carried out 'on-site', thus providing a rapid mastitis detection format.

Conclusions

Continuous monitoring of mastitis and its careful management is essential for the well-being of a dairy herd. This can be achieved through the detection of inflammation at its early stages and subsequently, the detection and treatment of the mastitis infection. Traditional and well-established tests include SCCs and culture-based methods. Assays mainly used 'on-site' are only indicative but not conclusive of the infection status of the animal. However, the development of novel analytical platforms incorporating enzymatic assays, immunoassays, biosensors and nucleic acid tests are progressively replacing the more conventional methods. Also, with advances in proteomics and genomics, new biomarkers are being discovered, allowing the disease to be detected at earlier stages. This will lead to assays with higher sensitivity, which can provide additional quantitative information on the level of inflammation 'on-site' and 'on-line' and which are also faster and less expensive. Furthermore, recent advances in microfluidics will facilitate the development of improved technologies that could subsequently be incorporated into automatic monitoring systems and portable assays for sensitive and rapid detection of mastitis.

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Practical methodology of some of the conventional and molecular methods

California Mastitis Test

The California Mastitis Test (CMT) is a rapid, accurate, cow-side test to help determine somatic cell counts (SCC) in a specific cow. The test was developed to sample individual quarters to determine the presence of subclinical mastitis. A cow with subclinical mastitis does not have abnormal looking milk or other clinical signs such as a swollen or painful udder. The test can also be conducted on bucket and bulk tank milk samples to help determine somatic cell counts (SCC) of the entire herd.

Principle

The accuracy of CMT is founded on 3 principles -

1. Leukocyte (WBC) numbers greatly increase when an injury or infection affects mammary tissue.
2. Leukocytes especially, polymorphonuclear leukocytes (PMNs) have large nuclei (DNA) compared to other cells or bacteria in milk.
3. Leukocte cell walls are mainly lipid (fat).

CMT reagent is a detergent with pH indicator added (reason for purplish colour). When milk and CMT reagent are mixed in equal amounts, the CMT reagent dissolves or disrupts the outer cell wall and the nuclear cell wall of any leucocyte, which are primarily fat (detergent dissolves fat). DNA is now released from the nuclei. DNA will string or gel together to form a stringy mass. As the number of leucocytes increase in a quarter, the amount of gel formation will increase in a linear fashion.

Materials Required

- Plastic paddle with 4 shallow cups marked as A, B, C, D.
- A bromocresol-purple-containing detergent (CMT reagent).
- Milk samples drawn from each quarters.

Procedure

1. Clean each teat with alcohol.
2. A small sample of milk (approximately ½ teaspoon) from each quarter is collected into a plastic paddle that has 4 shallow cups marked A, B, C and D.
3. An equal amount of CMT reagent is added to the milk.
4. The paddle is rotated to mix the contents. In approximately 10 seconds, read the score while continuing to rotate the paddle. Because the reaction disappears within 20 seconds, the test must be read quickly.

Advantages:-

Cheap, rapid, user friendly and can be used 'on-site' or in the laboratory.

Disadvantages:-

Can be difficult to interpret and has low sensitivity

Correlation between the CMT result and the somatic cell count

CMT score	Interpretation	Visible reaction	Total cell count (/ml)
0	Negative	Milk fluid and normal	0-200,000 0-25% neutrophils
T	Trace	Slight precipitation	150,000-500,000 30-40% neutrophils
1	Weak positive	Distinct precipitation but no gel formation	400,000-1,500,000 40-60% neutrophils
2	Distinct positive	Mixture thickens with a gel formation	800,000-5,000,000 60-70% neutrophils
3	Strong positive	Viscosity greatly increased. Strong gel that is cohesive with a convex surface.	>5,000,000 70-80% neutrophils

Reference

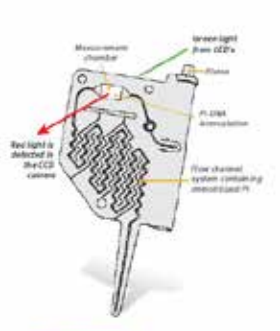
California Mastitis Test (CMT): An Invaluable Tool for Managing Mastitis by Roger Mellenberger, Dept. of Animal Sciences, Michigan State University (www.immucell.com /pdf/ an%20Invaluable%20Tool.pdf).

Somatic cell count using Nucleocounter SCC 100

The NucleoCounter SCC-100 offers unique ease of use and effective determination of the number of somatic cells in a milk sample. NucleoCounter® SCC-100 is based on the fluorescence microscopy and on the use of special, very practical, disposable cassettes, which dose the milk sample and put it in contact with the built-in, pre-dosed reagents.

Principal

Milk somatic cells are lysed using a lysis buffer and the DNA is liberated. Propridium Iodide is immobilized in the interior of the disposable SCC-Cassette. When the cassette is been loaded with the cell lysate the PI is dissolved and the cellular DNA is stained. After placement in the NucleoCounter the stained mixture is automatically transferred to the measurement chamber. Green light excites the PI-DNA intercalation and the red light emitted is registered in the CCD camera for correlation into a cell count.



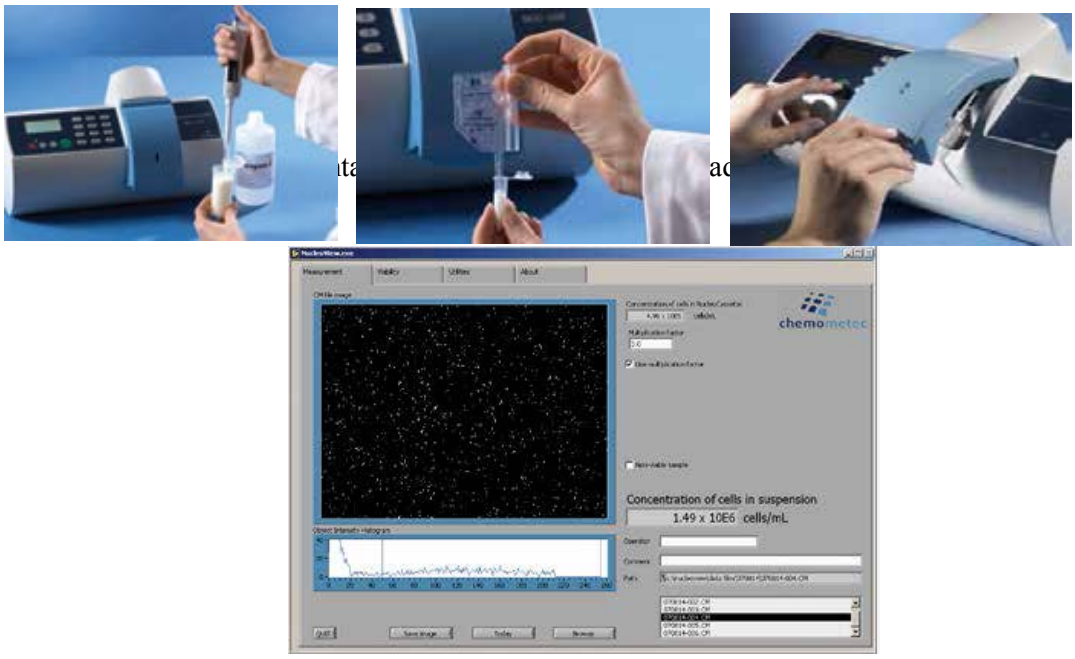
Materials required

- Nucleocounter SCC-100
- SCC cassettes
- Lysis Buffer (Reagent C)
- Milk samples drawn from each quarters

Procedure

1. Mix equal amount of milk sample and Reagent C (1:1 dilution), followed by inversion of the sample preparation tube to assure mixing.

2. Load the SCC-Cassette with the diluted milk sample by immersing the tip of the cassette into the solution and pressing the piston.
3. Place the SCC Cassette in the instrument and press the "Run" key. After 30 seconds the somatic cell count is presented on the instrument display and printed on the external printer. Optionally data is transferred to an external PC using USB connection.



Molecular detection of mastitis pathogens from milk

Bacterial DNA extraction from milk

Materials required

- Centrifuge, microcentrifuge tubes
- Sterilized saline solution (NaCl 0.9%)
- Lysis buffer (3M Guanidine thiocyanate, 20 mM EDTA, 10 mM Tris-HCl (pH 6.8). 40mg/ml Triton X-100, 10mg/ml DL-dithiothreitol)
- Binding solution (40mg/ml silica (Sigma Aldrich) suspended in lysis buffer
- Washing solution (25% absolute ethanol, 25% isopropanol, 100mM NaCl, 10mM Tris-HCl, pH 8)
- Elution buffer (10mM Tris-HCl, pH 8.0, 1mM EDTA)

Procedure

1. Dilute 500µL of milk sample with 500µL of sterilized saline solution (NaCl 0.9%) and centrifuge for 15 min at 600× g at 4°C; discard the supernatant. Repeat the step once.
2. Add 300µL of lysis buffer and 200µL of binding solution to the pellet resuspended in 50µL of saline solution. Mix and incubate for 5 min at room temperature. Centrifuge for 30 s at 450× g and discard the supernatant.
3. Add 200µL of lysis buffer and mix well. Centrifuge for 30 s at 450× g and discard the supernatant. Repeat this step once.
4. Add 200µL of washing solution and mix well. Centrifuge for 30 s at 450× g and discard the supernatant. Repeat this step once.
5. Add 200µL of absolute ethanol solution and mix well. Centrifuge for 30 s at 450× g and discard the supernatant. Vacuum-dry the pellet in an Eppendorf heat block for 10 min.
6. Add 100µL of elution buffer, resuspend the pellet, and incubate for 15 min at 65°C. Centrifuge for 5 min at 450× g and transfer the supernatant in a clean tube. To increase the DNA yield, a second elution step (with 5 min heating) may be performed.

Multiplex PCR for detection of five important *Staphylococcus* spp. (Shome et al., 2012)

Materials required

Equipments / plastic ware

- Thermal cycler
- Micropipettes
- PCR tubes

Reagents

- Oligonucleotide primers: Primers synthesized and supplied in lyophilized form are to be reconstituted to 100 µmol/µl stocks in nuclease free water (NFW).

Organisms	GenBank Accession no.	Gene	Primer designation	Oligonucleotide primer (52 - 32)	Location within gene	Amplicon size (bp)	Annealing temperature (°C)
S. chromogenes	AJ343945	*sod*A	SCHS1F	GCG TAC CAG AAG ATA AAC AAA CTC	134–157	222	58
			SCHS1R	CAT TAT TTA CAA CGA GCC ATG C	355–334		
S. haemolyticus	EU652775	*sod*A	SHS2F	CAA ATT AAA TTC TGC AGT TGA GG	42–64	531	58
			SHS2R	GGCCTCTTATAGAGACCACATGTTA	572–548		
S. epidermidis	CP000029	*rdr*	SERF	AAG AGC GTG GAG AAA AGT ATC AAG	400016–400039	130	56
			SERR	TCG ATA CCA TCA AAA AGT TGG	400145–400125		
S. sciuri	EU659914	*gap*	SSCGF	GAT TCC GCG TAA ACG GTA GAG	122–142	306	56
			SSCGR	CAT CAT TTA ATA CTT TAG CCA TTG	427–404		
S. aureus	X68425	23S r	SAS2F	AGCGAGTCTGAATAGGGCGTTT	678–699	894	56
		RNA	SAS2R	CCCATCACAGCTCAGCCTTAAC	1571–1550		

sod A: superoxide dismutase, *rdr*, ribonucleoside-di-phosphate reductase; *gap*, glyceraldehyde-3- phosphate dehydrogenase.
A: American type culture collection (ATCC). SAS2, *S. aureus*, SCHS1, *S. chromogenes*; SER, *S. epidermidis*; SHS2, *S. haemolyticus*; SSCG, *S. sciuri*.

- dNTP (10 mM)
- *Taq* DNA polymerase (1 U/µl)
- PCR buffer (10X)
- $MgCl_2$ (25mM)

PCR reaction

A 25µl reaction mixture is to be prepared in PCR tube. The PCR reaction mixture includes.

PCR buffer (10X)	:	2.5 µl
dNTP (10 mM)	:	0.5 µl
$MgCl_2$ (25mM)	:	2.0 µl
S. epidermidis forward primer	:	0.1 µl
S. epidermidis forward primer	:	0.1 µl
S. chromogenes forward primer	:	0.125 µl
S. chromogenes forward primer	:	0.125 µl
S. sciuri forward primer	:	0.1 µl
S. sciuri reverse primer	:	0.1 µl
S. haemolyticus forward primer	:	0.1125 µl
S. haemolyticus reverse primer	:	0.1125 µl
S. aureus forward primer	:	0.0875 µl
S. aureus reverse primer	:	0.0875 µl
Taq DNA polymerase (U/µl)	:	0.3 µl
Template DNA (50-100ng)	:	4.00 µl
Nuclease free water	:	14.65 µl
Total volume	:	**25.00 µl**

The DNA amplification should be carried out by keeping PCR tube along with reaction mixture in a thermal cycler using following conditions.

Steps	Temperature	Duration	No. of cycles
Initial denaturation	94°C	5 min	1
Denaturation	94°C	30 sec	30
Annealing	60°C	30 sec	30
Extension	72°C	45 min	30
Final extension	72°C	5 min	1

Agarose gel electrophoresis

Equipments

- Weighing balance
- Horizontal electrophoresis apparatus with power pack
- Microwave oven
- UV transilluminator/ Gel documentation system

Reagents

a. Agarose

b. TBE buffer (Tris - Borate EDTA buffer) (10X, pH 8.2)

Tris base : 108.0 g
Boric acid : 55.0 g
EDTA disodium salt : 8.3 g
Double distilled water up to: 1000.0 ml

Autoclave the solution and prepare 0.5X working solution

c. Gel loading dye (6X)

Bromophenol blue : 0.25% (w/v)
Xylene cyanol : 0.25% (w/v)
Sucrose : 40% (w/v) in distilled water

Store at 4°C before and after use.

d. Ethidium bromide (10 mg/ ml)

Ethidium bromide (Biogene, USA): 100 mg
Double distilled water : 10 ml

Stir thoroughly to ensure proper mixing. The container has to be wrapped in aluminum foil and stored at 4°C until use.

Procedure:

1. Assemble the gel casting tray by sealing the edges using adhesive tape. Place an appropriate comb to form a sample slot in the gel.
2. Prepare agarose solution by dissolving required quantity of agarose in a proportionate volume of 0.5X TBE buffer and melted in a microwave oven for 1-2 minutes (For preparing 1.5% agarose gel, 1.5g agarose in 100 ml of 0.5X TBE buffer)
3. Allow the molten gel to cool, add 0.5 µg of ethidium bromide and mix thoroughly by gentle swirling and pour into the gel casting tray. Avoid formation of air bubbles and allow the gel to solidify.

4. Place the gel casting tray in the electrophoresis tank after removing the adhesion tapes and add the electrophoresis buffer (0.5X TBE) to cover the gel to a depth of 1 mm. Remove the gel comb.
5. Mix 5µl of PCR product with 1µl of 6X gel loading dye and slowly load into the slots of submerged gel using a micropipette.
6. Close the gel tank and attach the electrical leads, so that the DNA will migrate towards the anode. Set the current at 100V and allow it to run for 40-45 minutes.
7. Following electrophoresis, the gel can be visualized under UV light with the Gel Documentation system.

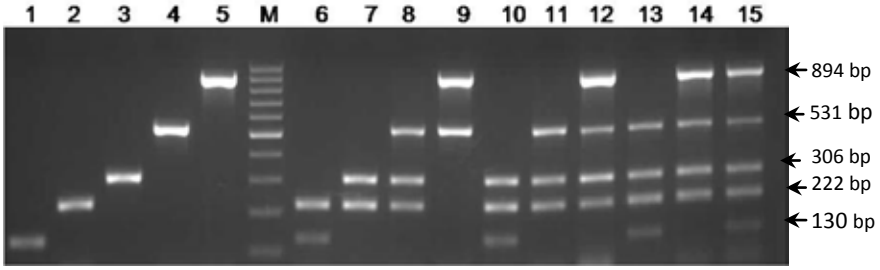


Fig. Multiplex PCR for detection of *Staphylococcus* spp. Lane1 to 5 represents: *S. epidermidis* (130bp); *S. chromogenes* (222bp); *S. sciuri* (306bp); *S. haemolyticus* (531bp); *S. aureus* (864bp); Lane M: 100bp DNA marker; Lane 6: Combination of *S. epidermidis* and *S. chromogenes;* Lane 7: Combination of *S. chromogenes* and *S. sciuri;* Lane8: Combination of *S. chromogenes, S. haemolyticus* and *S. sciuri;* Lane 9: *S. haemolyticus and S. aureus;* lane 10: Combination of *S. epidermidis, S. sciuri* and *S. chromogenes;* Lane 11: Combination of *S. chromogenes, S. haemolyticus* and *S. sciuri;* Lane 12*:* Combination of *S. chromogenes, S. aureus, S. haemolyticus* and *S. sciuri;* Lane 13: Combination of *S. chromogenes, S. epidermidis, S. haemolyticus* and *S. sciuri;* Lane14: Combination of *S. chromogenes, S. aureus, S. haemolyticus* and *S. sciuri;* Lane 15: Combination of *S. chromogenes, S. aureus, S. epidermidis, S. haemolyticus* and *S. sciuri.*

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Economic Impact of Bovine mastitis: Concepts and Methods

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Introduction

Livestock health economics is developing as a new discipline and is progressively developing a solid framework of concepts, procedures and data to support the decision-making process in optimizing animal health management. Research in this field primarily deals with three interrelated aspects, viz.

(i) quantifying the economic impact of animal disease;
(ii) developing methods for optimizing decisions when individual animals, herds or populations are affected; and
(iii) determining the profitability of specific disease control and health management programmes.

The productivity of livestock is influenced by many factors such as nutrition, animal health, genetic potential, environmental condition and herd management. The constraints do not act independently on the system. After nutrition, animal health tends to be the most serious impediment to production. Livestock development programmes cannot succeed unless a well organized animal health service is built up and protection of livestock against diseases and pests particularly against the deadly infections is assured. The consequences of animal diseases in livestock can be complex and generally go well beyond the immediate effects on affected producers. These diseases have numerous impacts, including:

- Productivity losses for the livestock (e.g. production losses, cost of treatment,)
- Loss of income from activities using animal resources (in such sectors as agriculture, transportation)
- Loss of well-being of human beings (morbidity and even mortality rates, food safety and quality)
- Prevention or control costs (production costs, public expenditure)
- Suboptimal use of production potential (animal species, genetics, livestock practices)

Background on Mastitis

Mastitis is a multietiological complex disease, which is defined as inflammation of parenchyma of mammary glands and is characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues. Mastitis occurs throughout the world wherever dairy cows are reared. The continuing presence of the disease may be attributed to deficient management, improper milking procedures, faulty milking equipment, inadequate housing, and breeding for ever-increasing milk yield. Several management and environmental factors must interact together to increase exposure of cows to mastitis organisms. The occurrence of disease is an outcome of interplay between the infectious agents and management practices stressing the defense of udder.

Losses due to Mastitis - Review

Mastitis has been and continues to be recognized as one of the major disease problems concerning the dairy industry. It is also one of the most costly diseases confronting the dairy farmer. Estimating economic losses resulting from mastitis becomes an extremely difficult task because of the many levels of infection and other factors. Mastitis is a global problem as it adversely affects animal health, quality of milk and economics of milk production and every country including developed ones suffer huge financial losses (Sharma et al., 2007). Mastitis, the most important deadly disease of dairy animals is responsible for heavy economic losses due to reduced milk yield (up to 70%), milk discard after treatment (9%), cost of veterinary services (7%) and premature culling (14%) (Bhikane and Kawitkar, 2000).

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

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

The annual losses per cow from mastitis in the United States in 1976 were estimated to be $117.35 and losses of milk yields caused by mastitis were 386 kg/cow per year and losses of discarded milk 62 kg/cow per year (Blosser, 1979). While these losses increased upto $185 to $200 per cow per year (Costello, 2004). Wilson et al. (2004) at Cornell University showed that clinical mastitis tends to strike high producing animals in second-plus lactation. In other words mastitis often hits the cows with the highest production potential, which expands the loss due to mastitis. According to the study, the estimated loss following clinical mastitis was almost 700 kg for cows in first lactation and 1,200 kg for cows in second or higher lactation (Wilson et al., 2004).

A study by Kossaibati and Esslemont (2000) in UK reported that, on average, 10% of cows with mild mastitis are culled. In severe cases the risk of culling is assumed to be at least 20%. A cull cow is assumed to cost £420 per cull, and the cost of a fatality is £1251. The National Mastitis Council of US during 1996 estimated the loss due to mastitis and the results are as follows

Sources of loss	Losses per cow ($)	Per cent to total (%)
Reduced Milk Yield	121.00	66.0
Discarded milk	10.45	5.7
Replacement cost	41.73	22.6
Extra labour	1.14	0.1
Treatment	7.36	4.1
Veterinary services	2.27	1.5
Total	184.40	100.0

Economic Models

The major challenge in assessing the actual impact of animal diseases at any level is the lack of understanding and clarity on the part of the researcher to decide about the parameters to be estimated which are generally grouped as direct and indirect impact indicators. They vary from disease to disease, and from species to species. Hence, so single or standard approach would suffice for the purpose.

Economic impact of animal diseases can be divided into six major parts, viz. production effects, market and price effects, trade effects, impacts on food security and nutrition, human health and environment, and financial costs. The immediate disease shock receives the most direct attention in impact studies because most often, it originates at the production phase. Yet, more far-reaching factors are sometimes overlooked. Disease impacts are generally easy to identify but may be difficult to quantify. In livestock, for example, delays in reproduction result in fewer offspring, which has long term effects not

easily measured in the present state. Even though the disease can be managed optimally by producers when the perceived economic damage is high, some level of disease if often accepted by managers when control is sufficiently costly. Still, researcher concludes that producer incentives for disease management can be changed through new technologies that lower the cost of prevention or control, subsidies or cost sharing of control measures, or on the consumer side, a change in public desire for disease risk-free products that changes relative prices. In short, livestock industry leaders would agree that disease outbreaks often have broader, long-term multiplier effects that extend beyond principal markets. Understanding the extent of such effects is an important element in measuring the potential costs and benefits of public policy tools to manage animal disease.

Sources of data

Two main sources of data on mastitis are (i) the places where the bovines are reared and (ii) the places where the records relating mastitis are compiled. The two sources are actually called as prospective and retrospective sources, respectively. The prospective sources include herd/flock/farm household, milk collection centres/cooperative societies, and livestock markets. The retrospective source includes veterinary clinic, diagnostic laboratories, artificial insemination centre and government departments. As most of the livestock are confined to rural area, the data from the prospective source and that too from the household maintaining the livestock would be more reflective and worthwhile.

The economic losses due to mastitis in livestock are broadly put into two main classes, viz. mortality losses and morbidity losses. The mortality losses include the value loss due to death of an adult animal. The morbidity losses can be grouped into four major classes, viz. production loss, treatment costs and miscellaneous costs. The data on the various physical and economic aspects mentioned are necessary to estimate the economic losses due to diseases in livestock.

The models used in evaluation of economic impact of mastitis can be grouped under two headings namely statistical/epidemiological models and economic models. Statistical models are used to identify the factors that contribute to the development of disease conditions, the magnitude and direction of the contribution, and relationships between diseases. Common models in this category include regression analysis, path analysis, discriminant analysis and analysis of variance. After the causes of a disease have been identified, disaggregated and quantified, the next step is to attach monetary value to the quantified impacts. Economic models used for this purpose are equi-marginal principle, partial budgeting, cost-benefit analysis, decision analysis, linear programming, Markov chains, systems simulation and dynamic programming.

Economic Models

The most common model that is used for assessing the economic impact of animal disease in our country is cost benefit analysis. Costs were defined as those expenditures relating to mastitis incurred by both government as well as producers for mastitis prevention and/or treatment. From farm surveys, it is possible to collect information on the expenditure meat by the farmers towards treatment and loss they suffered due to milk yield reduction, damaged milk etc. All those components comprise the direct economic loss suffered by the farm producers due to Mastitis

Following formulae could be used for estimating the impact on individual symptoms or signs or negative effects due to mastitis in bovines.

Loss due mastitis (L_m)

The loss due to mastitis comprise of Production Loss (PL), Treatment Loss (TL) and Depreciation Loss (DL).

It is denoted by

$$\mathbf{L_m = PL + TL + DL}$$

Production loss per sick animal (PL)

$$PL = N_{MD} \text{ X } (M_{\text{Pre-M}} - M_{\text{Post-M}}) \text{ X } P_M$$

Where,

PL = Production Loss per animal (Rs.)

N_{MD} = Number of mastitis days in the infected animal

$M_{\text{Pre-M}}$ = Milk Yield during pre-Mastitis period (litres/day)

$M_{\text{Post-M}}$ = Milk Yield during post-Mastitis period (litres/day)

P_M = Price of milk per litre (Rs.)

Production loss in a population

$$PLP = \sum_{i=1}^{n} SP \text{ X } IR \text{ X } PL$$

Where,

PLP = Production loss in a population (Rs.)

SP = Susceptible population (No.)

IR = Incidence Rate

PL = Production loss per infected animal (Rs.)

i=1 to n = Different species of animals

Loss due to treatment of sick animals (TL)

The instant loss to the farmer because of mastitis infection in his animal(s) is the cost of treating his ailing animal(s). This cost included the cost of medicines, veterinarian's fee and the cost of additional labour that might be required to provide extra care to the sick animals. That is, Treatment costs (LT) is equal to:

$$L_T = (C_P * N) + C_I$$

$$C_P = F + M$$

Where,

L_T = Treatment cost per infected animal (Rs.)

C_P = Cost of professional treatment (Rs.)

F = Fees for veterinarians / visit (Rs.)

M = Cost of medicines / visit (Rs.)

N = No. of visits to animal health services

C_I = Cost of indigenous treatment during the infected period (Rs.)

Treatment loss in a population

$$\mathbf{TLP = \sum_{i=1}^{n} SP \text{ X } IR \text{ X } L_T}$$

Where,

TLP	= Treatment loss in a population (Rs.)
SP	= Susceptible population (No.)
IR	= Incidence Rate
PL	= Treatment cost per infected animal (Rs.)
i=1 to n	= Different species of animals

Depreciation loss (DL)

$$DL = \sum_{i=1}^{n} TIP \times FR \times DMV$$

Where,

DL	= Depreciation loss (Rs.)
TIP	= Total Infected quarters in a Population (No.)
FR	= Fibrosis Rate
DMV	= Decrease in market value of the infected animal due to loss of each quarter (Rs.)
i =1 to n	= Different species of animals

Total Infected Quarters in the population (TIP) = Susceptible population X Incidence rate X Average number of quarters infected per animal

Fibrosis Rate (FR) = Number of quarters resulted in fibrosis/Number of quarters affected

Besides these components the opportunity cost of farm owner and laborers should also be included in estimating the total loss due to mastitis.

Conclusions

Bovine mastitis is one of the important production diseases of dairy animals which directly or indirectly affect the economy of the farmers ultimately affect the economy of the country. Controlling bovine mastitis in developing countries requires a considerable effort since most of the farmers in these countries rear animals in primitive conditions. The risk factors such as management practices (shed and udder hygiene, poor teat condition, poor environmental hygiene, sanitation, large herd size), host (breed susceptibility, higher yielder, udder immunity teat lesions) and diet (Cu, Co, Zn. Selenium and Vitamin E deficiency) has to be holistically addressed to mitigate the mastitis incidence. The economic impact assessment will be helpful for prioritizing the disease control measures at the national and sub-national level. It also helps in assessing the benefits to different stakeholders in upstream and down-stream due to implementation of control programme.

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Judging and selection of Dairy Cattle

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Judging and selection of cattle is an art that is acquired by constant association and familiarity with cattle and getting used to differentiate desirable characters from undesirable characters visual examination and judgment and if supported by pedigree and performance records and tests, is the best method of judging livestock.

The first step in judging livestock is to know the names of the different parts of the body of the animal. Next the relative importance of these points has to be noted. For each class of livestock, a score card indicating relative importance of each of these points has been prepared. In the scored card, marks are allotted for each of these points and the total is kept at 100.

Dairy cattle, especially cows should not only be economical producers but also satisfactory in appearance.

The following are the desirable and undesirable characters in dairy cattle, which should form a basis for proper selection and judging.

DAIRY COW UNIFIELD SCORE CARD

S. No	Particulars and desirable characters	Undesirable
	Attractive individuality, with feminity, weak, narrow faces; Vigour, with harmonious blending of all brightness, shallow Parts and impressive style and carriage nostrils small and tight	Short thick head Eyes small lacking Jaw at the base
1.	SHOULDER BLADES – (10) set smoothly forwards or And tightly against the body narrow. (Rump) BACK- straight and strong; LOM – Broad and nearly level; RUMP – long, wide and nearly level from hook bones to pin bones. Thurls high and wide apart. Tail slender and set level with back line and free from coarseness'	Sloping towards rear Sides; short and
2.	HEAD: (10) Breed characteristics. Clean cut and proportionate to body. Muzzle broad with large, open nostrils. Jaws strong; eyes large and bright; forehead broad and moderately dished; bridge of the nose straight, ears of medium sized and alertly carried	
3.	LEGS AND FEET: (10) strong straight legs pasterns Placed squarely; feet short; compact and well knees too close Rounded with deep heel and level sole Hind legs nearly perpendicular from hock to pastern from side view and straight from rear view.	Long legs with weak Standing regular
4.	2. DAIRY CHARACTERS – 20 Marks: Evidence of milking ability, angularity without weakness and freed from coarseness. NECK – long, lean and blending smoothly refinement. Into shoulders; clean cut throats; dewlap and Brisket; withers – sharp RIBS – well sprung, wide apart and rib bones should be wide enough it should be flat and long.	Lack of angularity and

	FLANKS – Deep and refined. THIGHS – Incurving to flat, strong, wide apart from the rear view providing sample room for udder and its rear attachment. Well defined triple wedges: TOP WEDGE – SIDE WEDGE – FRONT WEDGE – Give one regard to the period of lactation	
5.	BODY CAPACITY – 20 Marks: Relatively large in proportion providing sample capacity, strength and vigor. (10) BARREL – strongly supported, long and lack of depth; Deep; ribs widely sprung; width of the barrel straight ribs. Increasing towards (forming side wedge) (10) HEART GIRTH – large and deep well sprung fore ribs blending into the shoulders; wide chest floor.	Narrow barrel and Narrow chest
6.	MAMMARY SYSTEMS – 30 Marks: A strongly attached well balanced capacious udder of fine texture indicating heavy production and longevity. UDDER: Symmetrical moderately long, wide pendulous; hard with and deep; strongly attached soft; pliable and tissue. Well collapsed after milking; quarters evenly balanced. Fore and udders strongly attached. TEATS – uniform in size and placed symmetrically. MILK VEINS – Large, long, tortuous and with few Branching	Small size Humps in udder Short, small veins Branches

Selection of dairy cows with special reference to udder conformation

Choose cows with sound durable udders

If an udder is too small it may lack in capacity. Good texture can, however, compensate to a considerable degree for a deficiency in size. If an udder is too large it is subject to injury and often has poor texture. The most satisfactory method to be used in judging the soundness and condition of an udder is to observe it before milking and again after milking. In the examination of an udder in this way, it is important to know when the animal was last milked. It is possible then to estimate how much of the size is due to the milk contained and how much of the tissues of the gland itself. On milking out the udder should be reduced materially in size and the texture should be soft and spongy.

Age is an important factor in the development of an udder. Young cows cannot be expected to show the development in this organ that is found in mature animals. Lack of size, if the texture is good, in a fist-calf heifer is not as serious an objection as the same degree of deficiency in an older cow.

It has been demonstrated by numerous experiments that 55-65 percent of the yield of milk in normal animals is the product of the two rear quarters. A desirable balance is shown when not more than this portion of the milk comes from the rear quarters. A greater differnce than this between the front and rear quarters usually means that there is a lack of balance or an unevenness in the development between these two parts of the udder. The two front quarters and the two rear quarters should tend to match each other in size and development.

See that the udder is held snugly to the body:

It is important that the udder be attached high and wide at the rear. When it is attachéd high and wide at the rear there seems to be fewer tendencies for the tissues to relax and permit the udder to break away from the body. Furthermore, an udder with a high, wide rear attachment is usually wider than one with a narrow attachment and so has good capacity without extreme depth. The front attachment of the

udder should extend well forward and be slightly wider than the rear attachment. There should be no tendency for a separation between the udder and the body of the animal. The tendency for an udder to "break away" from the body becomes more pronounced with each succeeding lactation.

Perhaps the most serious fault that an udder can have, insofar as attachments are concerned, is the failure of the median or central support. The median suspensory ligament provides the principal support for the udder. If it fails, center of the udder drops down, the teats, instead of pointing directly downward, point outward, and the udder is badly misshapen. Furthermore, when this condition is pronounced, machine milking is done with great difficulty, and the cow losses most or all of her usefulness in her.

Examine the udder for texture and quality

It is highly desirable that the udder be of good texture or, in other words, contains a high percentage of secreting tissue. Such an udder is capable of producing more milk per given unit of tissue than one that possesses a higher proportion of connective tissue. It is almost impossible to judge the texture when an udder is highly distended with milk. When this happens, it will be necessary to remove the milk and examine the empty udder. An udder that collapses when milked out and is loose and pliable when handled is ordinarily one of good texture. Udders that are hard and "meaty" to the touch are often associated with a lack of persistency in the cows.

Observe whether or not teats are well placed and of proper size

The teats are well located on the udder when set at least six inches apart from front to rear and almost an equal distance apart from side to side. Teats that are too close together interfere with the operation of milking. Teats should be large enough to be grasped readily by the hand and yet not so large as to make milking difficult. Teats that are too small make machine milking somewhat difficult and hand milking slow and tiresome.

Examine teats for warts, extra openings, or leakage

The teat should be smooth and free from warts. If warts are present, they can sometimes be reduced and softened by the application of Vaseline, sweet oil, or some similar substance. Occasionally there is an extra opening in the side of a teat. Not infrequently a cow is found whose sphincter muscle, which ordinarily closes the opening of the teat, does not function normally. The result is that the milk drips, or at times may even run in small streams, from one or more teats. This condition is undesirable even though cows so affected are usually easy milker.

Notice development of milk veins and wells

Perhaps no two terms are more frequently misunderstood than "milk vein" and "milk well". The name "milk vein" and "milk well". The name "milk vein" would seem to indicate that the vein contained and carried milk. This is not true. The milk vein carries blood from the udder toward the heart, for purification. It thus performs the same function as other veins, all of which carry blood to the heart. Two milk veins are located on either side of the animal just in front of the udder and extend along the underline beneath the skin. The openings through which these veins enter the body cavity are termed milk wells. Generally there are but two openings, or wells, one on either side. Not infrequently the milk vein separates into a number of branches, each one of which enters the body cavity through a separate opening. Cases are reported in which as many as thirteen openings, or milk wells, have been observed in a single animal.

Large milk veins and milk wells are associated with high milk yield. It must be borne in mind, however, that extreme development usually appears in older animals. Large milk veins and wells are more an evidence that cows have produced heavily in the past than that they will produce well in the future.

It should be recognized that the milk vein, the subcutaneous vein that you can observe on the outside body, is not the only one that can carry blood from the udder to the heart. Two internal veins on each side, generally smaller in size than the external veins also provide exits for the venous blood.

Therefore, it is not so essential that the external veins be large to insure an adequate blood flow through the udder.

Use production records whenever possible to aid in making selections

It is highly desirable to know how to choose the right kind of a dairy cow, but an operator should obtain all of the valid evidence possible when selecting milking animals. Authentic production records such as those kept by the Dairy Herd Improvement Association, the Herd Improvement Registry, or the Advanced Registry present proof of productive capacity. It should be recognized, however, that the conditions under which records are made have a definite influence upon the size of the record. Such variables as age at time of calving number of milking per day, length of previous dry period, whether or not a calf was carried during the major part of the lactation, kind and quantity of feed, and nature of management are all factors that contribute to a lactation record. Add to these major genetic influences such as lactation drive and percent fat content of milk, and it should be apparent that production records require some interpretation as to their real meaning.

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Role of genomics on mastitis resistance in dairy animals

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The incidence of infectious diseases impedes the growth of livestock industry, despite widespread application of antibiotics, vaccines, quarantine and other conventional disease control measures. An alternative control of infection in cattle is genetic disease resistance, which is the inherent capacity of a previously unexposed animal to resist a virulent challenge. Genomics has opened a new vista in disease investigation and vaccine research in livestock. So far, genetic selection of animals placed emphasis on productivity and efficiency which has potentially reduced natural disease resistance. Candidate genes having direct or indirect association with SCC and occurrence of mastitis and sub clinical mastitis which are of paramount importance. Hence, it is high time to implement these combined efforts of genomics with conventional breeding practices for selection of superior dairy animals to bring cost effective and faster genetic improvement.

Selection of superior dairy animals based on true genetic merit or breeding value is the main key for their genetic improvement. Genetic improvement depends on two principal components *viz.*, (a) the existence of variability in the population (b) the population which heritable, i.e. the heritability. Success for selection also depends on having clear, well defined objectives which support selection through replacing less desirable alleles by better alleles in the population. For the most simple form of selection called "mass selection" or selection on phenotypes measured on individuals in a population which is expressed as follows:

$$\Delta G = ih^2O\text{-}_p$$

The present equation is that genetic progress is the product of intensity of selection times the heritability of the trait under selection times the phenotypic standard deviation of that trait. If any of these three parts is low, genetic progress through selection will be slow. The economic value of the trait may still justify efforts to improve it through selection; as such improvement is a permanent change that benefits all future offspring. Heritability helps the dairy producer to decide which traits justify improvement through selection. Heritability is one important component of the equation used to predict genetic progress from selection to improve a trait

Variation:

In general, it is very difficult to change genetically the population due to the less variability in terms of additive gene action exploited by selection, dominance and epistasis – the most suitable components exploited by crossing and hybridization respectively.

Heritability:

The heritability or the ratio of the additive genetic variation to the total phenotypic variation is determined by the genetic configuration of the animal and it is fixed which provides an idea about the scope for expected genetic improvement in a population through selection. The higher the degree of heritability (h^2), it is easier to change the character or trait by selection.

Characterization of candidate genes and their role for resistance against mastitis in various cattle breeds:

Genes under MHC locus:

All most all vertebrates harbour a major histocomatibility complex (MHC) with a primary role in the immune system. This complex encodes for the class I and class II molecules, which are transmembrane glycoproteins composed of A and B chains. The class I molecules are expressed in all cell types, whereas, the class II genes are expressed only on selected cells of the immune system, such as macrophages, dendritic cells and certain B lymphocytes collectively known as antigen presenting cells (APCs).

The products of class II genes find antigenic peptides generated by proteolytic activities and present them to T-helper lymphocytes. The class II genes are highly polymorphic, the polymorphism being mainly located in the exon-2, which codes for extracellular domain forming part of the antigen recognition site (ARS). The three dimensional structure of the human DR-functional molecule depicts that the highly polymorphic nature of exon-2 is not random rather restricted to certain regions while other regions might be conserved. The polymorphism at this region is of functional importance as it reflects the immune capability of the organism. Various studies suggest that there is possible role of genes (DRB3, DQA and DQB) under MHC class II to control the disease resistance

***BoLA-DRB3* gene**

Bovine Lymphocyte Antigen *DRB3* (*BoLA-DRB3*), a gene of the Major Histocompatibility Complex (MHC) has received attention because this is considered to be a potential genetic marker associated with disease resistance traits in cattle. A study was conducted in 51 Deoni and 60 Ongole breeds of cattle to investigate genetic variations in *BoLA-DRB3.2* alleles. Results of present study indicate that the *BoLA-DRB3* exon 2 is highly polymorphic in both Deoni and Ongole breeds of cattle. The most common alleles in Deoni and Ongole breeds of cattle, alleles **6* (0.216) and **15* (0.225) had the highest frequency, respectively. The second highest number of allele *BoLA* alleles **11* and **6* which were present at a frequency of 0.167 and 0.200 in Deoni and Ongole breeds of cattle, respectively. Out of the 22 identified alleles detected in Deoni, nine alleles (*BoLA-DBB3.2*6, *11, *9, *15, *20 *23, *34, *47* and **51*) represented 78.4 per cent of total allelic frequencies. Whereas, in Ongole, out of the total alleles detected seven alleles (*BoLA-DRB3.2*15, *6, *12, *13, *23, *31* and **47*) represented 77.5 per cent. Direct sequencing *BoLA-DRB3.2* allele was carried out to get the complete picture of polymorphism at nucleotide level. The interesting feature noticed in Ongole breed was that at position 91 and 133 of the sequence, it had both A and G nucleotide in contrast to *Bos taurus* breed, which had only TT nucleotides. In these breeds of cattle, there were similar variations at positions 91, 133, 211, 232 and 256 was noticed which is due to unique nature of native breeds (Sarvanan, 2009).

NRAMP1 Gene:

The NRAMP1 gene is a major candidate gene influencing the outcome of infection with intracellular pathogens in numerous species. NRAMP1 gene is highly conserved in many mammalian species and the NRAMP1 gene shows considerable conservation in structure between mice and humans. Natural resistance associated macrophage protein 1 (NRAMP1), an integral transmembrane protein, is reported to influence the intraphagosomal microbial replication. Several studies indicated that the NRAMP1 locus plays a critical role to control the innate resistance or susceptibility of bovines to infections with a group of unrelated intracellular pathogens.

Malnad Gidda, the dwarf breed of cattle in Karnataka can withstand the harsh, hot and humid climate with heavy rainfall situation prevailing in Malnad region of Karnataka. In view of absence of any of incidence of infectious diseases in Malnad gidda cattle as revealed by our earlier surveys and lack of reports to the contrary, the NRAMP1 gene was investigated in this breed aiming at detecting polymorphism at exon V to VII of this gene employing PCR RFLP technique.

The study on polymorphic pattern of NRAMP1 gene with 951 bp region of exon V to VII through PCR RFLP using *Hae* III and *Alu* I restriction enzymes was carried out. *Hae* III restriction enzyme revealed that there was no polymorphism with PCR RFLP pattern. With the use of Alu I restriction enzyme, PCR RFLP pattern showed the presence of polymorphism in respect of NRAMP1 gene with 951

bp (exon V-VII). In this study, three genotypes *viz*., AA, AB, and BB with a frequency of 0.3, 0.475 and 0.225 respectively were detected. The allelic frequency of A and B alleles were estimated as 0.5375 and 0.4625 respectively. As the frequencies are in H-W equilibrium, it is inferred that there was no selective advantage of either of the alleles in respect of disease resistance.

Neutrophil β-defensins genes

Defensins, cysteine-rich antimicrobial peptides are mainly found in neutrophils and epithelial cells in mammalian species**.** Their broad-range antibiotic activity influences on host defense by eliminating or preventing the colonization of pathogenic organisms at a variety of anatomic sites. On the basis of the position and six conserved cysteine residues, defensins in vertebrates are divided into two categories viz., α and β-defensins. Unlike α-defensins which are produced by neutrophils and intestinal cells, β-defensins are primarily expressed by epithelial cells of the skin, kidneys and trachea-bronchial lining. These are either released upon microbial invasion and their regulation is stimulated by the lipopolysaccharide (LPS) and tumor necrosis factor-α (TNF-α).

In cattle, β-defensins are 3 to 6 KD β peptides which contain disulfides. They are encoded by a large gene family expressed in a wide variety of tissues. Thirteen β-defensins have been isolated from bovine neutrophils, although gene expression is restricted to mature myelopoietic cells. Other sites of β-defensins gene expression include the pseudo-stratified columnar epithelial cells of the trachea, squamous epithelial cells of the tongue and simple columnar epithelial cells of the distal small intestine and colon. To date, β-defensin gene expression has not been reported in macrophages, although defensins have been shown to exhibit antimicrobial activity against intracellular pathogens of macrophages. The mammalian epithelia provide the first line of defense between organism and environment. When this barrier is breached, microorganisms invade and acute inflammatory response occurs. The physical barrier is fortified by the secretion of numerous antibacterial agents, including immunoglobulin antibodies, enzymes viz., lysozyme and proteins viz., lactoferrin. Antimicrobial peptides were also detected in barrier epithelial cells of several mammalian species, including mice, cows and humans.

Although the expression of antimicrobial molecules in epithelia suggests that there is possible role of β-defensin genes to take part in the host defense mechanism. Because of the epithelial origin and secretary nature of the β-defensins, it seems that mastitis induced BNBD-4 and BNBD-5 expression in bovine mammary gland tissue, secretes defensins onto its surface and are highly expressed during initial stages of mastitis. Additionally, during lactation, secretion of defensins into milk may also protect the tissue surface from bacterial colonization and contribute to the feeding-mediated passive transfer of innate immunity from mothers to their breast-fed-infants. These genes are expressed during entire stages of mastitis. The association of BNBD-4 and BNBD-5 variation with major infectious disease provides support for the strategy of mapping and identifying genes for resistance to mastitis which may further help for genetic control of mastitis resistance and marker assisted selection in cattle and buffaloes.

BTN1A1

In *BTN1A1* Exon 3, the frequencies of Genotype CC and allele C were observed to be predominant in Deoni cattle, whereas the frequencies of Genotype AA and allele A were more prevalent in HF crossbred cattle. Statistical analysis using GLM procedure of SPSS 17 software revealed that the heterozygotic genotype AC was associated with lower SCC values in both the studied breeds, whereas AA genotype was significantly ($P \leq 0.05$) related to higher SCC.

In HF crossbreds, significantly ($P \leq 0.05$) higher SCC was observed during mid lactation stage, whereas in Deoni cows, no significant variations were observed in SCC values in relation to stage of lactation. The fat per cent was significantly ($P \leq 0.05$) higher during fifth parity in Deoni cattle, whereas no significant effect of parity on fat per cent was observed in HF crossbred cattle.

The results of the study indicated that in Exon 8, Genotype AA and allele A were related to more milk fat percent in both the studied breeds. The results of this study also indicate that Genotype AA (Allele A) in Exon 3 of *BTN1A1* gene was related to an increased milk SCC content in both breeds, whereas the

Genotype AA was related to more fat content in both the breeds. Thus the genetic variation in the bovine *BTN1A1* gene could be exploited as a marker for QTLs controlling milk yield and fat percentage as well as SCC).

TLR gene

Toll like receptors (TLRs) are multi gene family of pattern recognition receptors. These are highly conserved group of proteins which have been identified in organisms as diverse as insects and mammals. Within the mammals it is reported that TLR contains 10 members (TLR1-10). The TLRs provide the host with a means for discriminating foreign body from self and they function at the earliest stages of immune development. In addition to this, TLRs are able to specifically identify pathogenic agents from commensals. During IMI, mammary epithelial cells and tissue macrophages become activated via their pathogen recognition receptors, which recognizes molecular Signatures associated with invading pathogens, called PAMP (Pathogen Associated Molecular Pattern). Pattern recognition receptor-PAMP ligation induces transcription and secretion of cytokines and chemokines which involve and activate blood neutrophils followed by monocytes to the site of infection. These receptors recognize a great variety of PAMP and play a pivotal role in the initiation of inflammatory response and subsequent adaptive immune response to pathogens. Recognition of foreign molecules is based on the molecular patterns that are indicative of entities likely to cause harm to the host. There are limited published information on expression levels of bovine TLRs. The majority work is cofined to work on TLR2 and TLR4.

Mastitis is an inflammatory disease of the mammary gland caused by IMI. Somatic Cell Count (SCC) is used as an indicator to detect the occurrence of clinical and sub clinical forms of mastitis. Several indirect tests for sub clinical mastitis practiced under field condition need further verification for its confirmatory diagnosis through microbiological evaluation. Thus, it is necessary to observe the microbiological status of SCC positive samples collected from apparently health dairy cows. Literatures revealed that TLR 4 is involved in PAMP recognition; mutations in TLR 4 can compromise the host immune response to certain pathogens. Moreover, the TLR 4 is highly polymorphic and its expression is associated with IMI in bovines. Research reports revealed that the polymorphism of haplotypes of TLR 4 gene in high yielding HF animals is associated with somatic cell, the indicator of mastitis. Hence this gene may be a potential candidate for use in Marker assisted selection to enhance the mastitis resistance in dairy cattle.

In a study conducted at National Dairy Research Institute, Bangalore, milk samples were collected from 95 HF and 52 Jersey crossbred cows. Snapshot tests *viz*., Electrical Conductivity meter test, California Mastitis and Digital Somatic cell counter tests were applied. The range of somatic cell counts (SCC) per ml of milk varied from 85,000 to 30,00,000 in HF crossbred cows. The range of SCC in Jersey crossbred cows varied from 50,000 to 30,00,000 per ml of milk. Occurrence of mastitis observed in HF crossbred cows was 10 percent and subclinical mastitis as 30 per cent. Whereas, the mastitis was recorded as 21 per cent and subclinical mastitis as 54 per cent in Jersey crossbred cows. SSCP analysis for TLR 4 gene indicated that exon 1 and 3 of TLR4 have shown polymorphism and exon2 is highly conserved showing monomorphic pattern. From the investigation on TLR 4 gene polymorphism, it was concluded that the somatic cell count detected in BB genotype of exon 3.3were found to be lower in both HF crossbred and Deoni native breed of cattle. Hence, the possibility of udder infection in cattle with BB genotype are less than other genotypes, which suggests that the selection of dairy cattle with BB genotype may be performed for marker assisted selection for future breeding programme.

Genetic and environmental deterrents to breeding for disease resistance in dairy cattle

Selection for increased milk production in dairy cows has often resulted in a higher incidence of disease and thus incurred a greater health costs. Considerable interests have been shown in breeding dairy cattle for disease resistance in recent years. Limitations of breeding dairy cattle for genetic resistance include 1) complexity of disease resistance, 2) difficulty in estimating genetic parameters for planning breeding programme against disease, 3) undesirable relationship between production traits and disease, 4)

disease as affected by recessive genes, 5) new mutation of the pathogens, and 6) variable environmental factors.

The hidden problems for estimating genetic and phenotypic parameters involving disease incidence were examined in terms of categorical nature, non-independence, heterogeneity of error variance, non-randomness, and automatic relationship between disease and production traits. In light of these limitations, the prospect for increasing genetic resistance by conventional breeding methods would not be so bright as we assume.

Since the phenomenon of disease is the result of a joint interaction among host genotype, pathogen genotype and environment, it becomes essential to adopt an integrated approach of increasing genetic resistance of the host animals, manipulating the pathogen genotypes, developing effective vaccines and drugs, and improving the environmental conditions. The advances in DNA-based technology show considerable promise in directly manipulating host and pathogen genomes for genetic resistance and producing vaccines and drugs for prevention and medication to promote the wellbeing of the animals.

As a result of various complex factors affecting infection, heritability of disease traits is low, indicating a slow response to selection for disease resistance. Marker data are most useful for genetic evaluation in traits with low heritability. The use of markers or candidate genes as an aid to selection (marker-assisted selection) is effective particularly for lowly heritable and sex-limited traits. For traits that cannot be measured directly in either sex, *eg*. Disease resistance, traditional quantitative methods will be expensive because it would require sib or progeny tests. Marker-assisted selection is therefore most suitable for the improvement of disease resistance status.

Recent advances in molecular genetics have made it possible to screen favourable genetic markers associated with disease resistance. Molecular genetic information can be used to improve disease resistance in two ways: (1) marker-assisted selection and (2) marker-assisted introgression. Selection using linked markers can be effective and does not require the identification of the functional mutations, although some level of fine mapping is required. The detection of the functional mutation will improve the efficiency of selection and will increase our understanding of quantitative genetic variation and the relationships between traits. Somatic cell score is used as an indicator of resistance/susceptibility to mastitis. Literatures depicted that the 5'- *Alu* I marker of the bovine growth hormone receptor (GHR) was associated with the somatic cell score. Genes of the MHC are related to genetic resistance in cattle. These reports indicate that genetic markers could serve as a valuable tool to aid selection for disease resistance.

Marker assisted selection for disease resistance:

Marker is defined as any stable and heritable variant(s) that is detectable and measureable by a suitable method. This can be used to detect or identify the presence of a specific genotype or phenotype other than itself. The marker gene along the whole segment of chromosomes is inherited to the offspring from its parents. If a particular segment contains a gene or genes causing variation between animals for particular trait, then the association between the particular segment obtained by animal and its performance can be established. Therefore, progeny can be selected on the basis of chromosome segments inherited from their parents as well as their performance. Substantial progress has been made over the past decades through the application of tools of molecular genetics in identification of loci on chromosomal regions which influence traits of economic importance in Livestock production system. This has enabled opportunities to enhance genetic improvement programmes in livestock by direct selection on genes or genomic regions through marker-assisted selection and gene introgression.

Marker-assisted selection requires a three-phase development and testing programme. First, in a preliminary screening procedure, locations of QTL within the genome must be identified, using widely distributed markers in a reference population of known family structure. In the second phase, the presence and phenotypic impact of QTLs must be verified or validated in commercially relevant populations. Only after the location and effects of the QTL are validated one can ensure the third phase. Commercial application of marker-assisted selection in the population at large scale can be undertaken. For this purpose a heterogenous animal material, inclusion of different breeds as well as different physiological traits are to be selected and this panel of animals is scanned for genetic polymorphisms. Finally, a DNA

based micro array assay containing the most informative SNPs could be designed and implemented in breeding programmes.

Types of Genetic Markers:

Application of molecular tools for genetic improvement depends upon the ability to genotype individuals for specific genetic loci. For these purposes, three types of observable polymorphic genetic loci are generally distinguished: 1) direct markers: loci that code for the functional mutation; 2) LD markers: loci that are in population-wide linkage disequilibrium with the functional mutation; 3) LE markers: loci that are in population wide linkage equilibrium with the functional mutation in outbred populations. These marker loci differ not only in methods of detection, but also in their application in selection programmes. Whereas, direct markers and, to a lesser degree, LD markers, allow for selection on genotype across the population because of the consistent association between genotype and phenotype, use of LE markers must allow for different linkage phases between markers and QTL from family to family. Thus, the ease and ability to use markers in selection is opposite to their ease of detection and increases from direct markers to LD markers and LE markers. In nut shell, selection on these three types of markers are referred to as gene-assisted selection (GAS), LD markers assisted selection (LD-MAS), and LE marker-assisted selection (LE-MAS), respectively.

DNA Markers

DNA markers are those markers which are capable of detecting genetic variation at DNA level viz., Restriction Fragment Length polymorphism (RFLP), minisatellite or variable number of tandem repeats (VNTR), microsatellite, random amplified polymorphic DNA (RAPD), Detection of Single Nucleotide Polymorphisms (SNPs), Expressed sequence tag (EST) technologies (used to identify previously unknown genes), DNA chip technologies etc.

Use of Molecular Data in Selection:

In principle, all applications of molecular genetic information for genetic improvement involve selection on a molecular score, although the composition of this score differs from application to application. The molecular score is based on the presence or absence of certain alleles or genotypes, as in marker assisted introgression (MAI), or on estimates of marker or QTL effects, which can be summed over loci when multiple QTL regions are considerd for selection. In general, three strategies can be distinguished for the use of the molecular score (MS) in selection, in combination with phenotype, or expected breeding value (EBV) derived from phenotypic information.

Future Scope:

In India, under low input livestock production system it might be difficult to reallize the value of marker information as it would be harder and obviously more expensive to ascertain the linkage relationship in the case of linked markers. However, a QTL marker is likely to be more successful in an environment with intensive information on pedigree and performance records. Inclusion of genetic selection for disease resistant genotypes might be a beneficial addition to comprehensive programme of disease control. As a part of molecular tools for genetic improvement, gene intervention particularly for disease resistence genes in high yielding dairy breeds could be of great value to make them more tolerent in hot and humid climate. Identification of genetic markers for disease resistance can obviate the need for costly and hazardeous disease challenge testing. True genetic potential of cattle and buffaloes can be identified, uncomplicated by environmental effects in the assay system. Enhancing immune competence can improve vaccine efficacy and reduced need for antibiotic treatment, thereby reducing residues in milk or other animal produce. Desirable genotypes can be selected at an early age.

Advances in molecular technology including nano technology, pharmacogenomics, molecular vaccinology, stem cell research, proteomics, bioinformatics and computer assisted genome programme and whole genome approach will create much information for further improvement in disease resistance, drug discovery and marker assisted selection in livestock in future.

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Nutritional Considerations to Control Mastitis in Dairy Animals

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Mastitis is one of the main diseases that affect the profitability of dairy farmers. In financial terms, mastitis is the most costly disease in the dairy industry. These losses are a result of reduced milk production, discarded milk, replacement costs, treatment cost and veterinary service. It is now well known fact that the subclinical mastitis (SCM) is more serious and is responsible for much greater loss to the dairy industry. Amongst the many factors influencing profitable dairying, feeding accounts for about 60-70% of the total input and hence any improvement in the nutrition and feeding aspect of dairy cattle will have both qualitative and quantitative impact on health and productivity. Nutritional status has a profound direct as well as indirect influence on udder health of dairy animals. Majority of clinical mastitis are detected during the first month of lactation, so it is imperative that the immune system is functioning properly at this time of high stress for the cow. Animal needs to be maintained at good nutritional status for proper functioning of immune system. During the transition period of early lactation, the dairy cow can experience negative energy balance, which can have a major impact on the immune response. It is well known that cows in negative energy balance are at a higher risk of ketosis. Periparturient period is the most susceptible period for acquiring infections in dairy cattle. Metabolic disorders like milk fever, ketosis and rumen acidosis predispose the animal for infections of uterus and mammary gland. It is reported that cows with milk fever are 5 times more likely to get clinical mastitis than the normal. Clinical ketosis is associated with a 2- fold increase in the risk of clinical mastitis.

There are various genetic, physiological, and environmental factors that can compromise host defense mechanisms during the functional transitions of the mammary gland. Nowadays, the lactating cow has been genetically selected to produce more milk, which is the basis of the dairy industry. However this increase in milk volume metabolically stresses dairy cows and affects mammary gland immunity by impairing defense mechanisms and decreasing the resistance to mastitis. In addition, the milking procedure can cause trauma to teat and tissues, making it easier for the invasion and colonization of mastitis causing pathogens in the mammary gland.

Immunosuppression

In addition to the potential hepatic metabolic disorder associated with negative energy balance, periparturient dairy cows also undergo a period of reduced immunological capacity during the weeks around calving. The combined results of these dysfunctions are that dairy cows may be hyposensitive and hypo responsive to antigens, and therefore more susceptible to infectious disease such as mastitis. Ketosis may increase the risk of mastitis in periparturient immunosuppressed cattle because many immune cell types are negatively affected by metabolite levels typical of a ketotic environment (i.e., low concentrations of glucose and high concentrations of ketone bodies). A ketotic environment suppresses bovine lymphocyte blastogenesis, lowered the chemotactic capacity of leukocytes, decreases the bactericidal activity of neutrophils. Significant quantity of calcium is required for milk synthesis and inadequate calcium at the onset of lactation results in hypocalcaemia (milk fever). Although it is important for milk synthesis, calcium is also important for intracellular metabolism and signaling in most cell types, including the leukocytes of the immune system. Low blood calcium around the time of calving could contribute to periparturient immunosuppression. Vitamins and minerals, such as vitamin E, A, selenium, copper, and zinc, when properly supplemented can enhance a cow's immunity against mastitis.

Defences of mammary gland

The teat end is the first barrier against invading pathogens. The anatomical and physical characteristics of the teat canal (tightness of closure and keratin lining) inhibit penetration of udder pathogens. Approximately 40% of the keratin lining is removed at each milking and, therefore, it requires constant regeneration. Consequently, it is important to ensure that there is closure of the teat canal post-milking. After bacteria breach the teat end, they are taken up and destroyed by leukocytes and leukocyte chemotaxis is one of the major factors involved in migration of these cells towards the centre of inflammation cows experiencing negative energy balance show an impairment of udder defense mechanisms. Possible explanations for these effects are reduced capacity for phagocytosis by polymorphonuclear neutrophils (PMN) and macrophages, and decreased generation of chemoattractant for blood leukocyte migration into the infected gland.

Impact of nutrition on mastitis

Antioxidants and trace minerals play important roles in immune function, which inturn can influence some aspects of health in transition dairy cows. Vitamin A and Zn influence epithelial health, can impact physical defense barriers of the udder, and also alter the quality and quantity of the keratin plug. Phagocytic cells are influenced by a number of nutrients, including Cu, Zn, Se, and vitamins A and E. Copper can affect phagocytic function, with variable impacts on cell mediated and humoral immunity in cattle. Lymphocyte activity and antibody production can be influenced by energy, protein, Zn, and vitamin A, D and E.

Immunity

The ability of an animal to resist an infection depends upon the competency of its immune system. Among all the nutrients influencing the immune response, trace minerals and vitamins play a critical role. General concepts of micronutrient deficiency include (a) alteration in immune response occur early in the course of reduction in micronutrient intake, (b) the extent of immunological impairment depends upon the type of nutrient involved, its interaction with other nutrients (Vitamin A, & Zn; Vitamin E & Se), severity of deficiency and type of infection, (c) opportunistic infections (mastitis, cold, cough) are more common during a micronutrient deficiency (d) excess intake of micronutrients is also associated with impaired immune response. Important immuno modulating micronutrients include vitamin A and Zn, vitamin E and Se, Cu, Fe and B complex Vitamins (folic acid, B_6, B_{12}). Micronutrient deficiency is one of the most probable reasons for poor antibody response following antigenic/vaccine stimuli. Efforts are being made to use some of the micronutrients like Vitamin E and Se as adjuvant along with the antigen for enhancing the antibody response. Use of trace minerals in chelated form/organic form (Cu-Lysine, Zn-Methionine, Se-Methionine) and area specific mineral mixture are the other options.

Antioxidants

Oxidative stress is the basis of any disease development. Antioxidants are widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases. Antioxidants are molecules that slow or prevent the oxidation of other chemicals. Oxidation is a redox chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can involve the production of free radical, which can form dangerous chain reactions. Although oxidation reactions are critical for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidant molecules or inhibition of these antioxidant enzymes causes oxidative stress and may damage or kill cells. As oxidative stress has been implicated in the pathogenesis of many diseases, including mastitis and the use of antioxidants is intensively studied.

Vitamin E and Selenium

Vitamin E is a lipid soluble antioxidant that protects against lipid peroxidation initiated by free radicals and has been shown to play an important role in immune response and health of dairy cows Selenium is an essential component of the enzymes glutathione peroxidase and thioredoxin reductase located in the cytosol of the cells; which function in preventing oxidative stress. In addition, Se is also considered to have a protective effect on phagocytic cells from autoxidative damage during the respiratory burst. Leakage of free radicals from the phagolysosomes, or failure to detoxify these products, could affect the microbicidal and metabolic functions of phagocytic cells Studies have shown that supplementation of Se and vitamin E to dry cows reduced the duration of mastitis and incidence of clinical mastitis, with vitamin E alone being a good supplementation in preventing mastitis in early lactation. Vitamin E supplementation increases the blastogenic responses of both T and B cells, and that the antibody response was highest in calves supplemented with 125 IU vitamin E per day. Injecting 3000 IU of vitamin E subcutaneously, 10 and 5 days before expected calving, significantly increased the intracellular kill of bacteria by neutrophil at calving. Selenium and vitamin E supplementation reduced mammary gland infections by 42% and reduced clinical mastitis by 32%.

Copper and zinc

Copper has an important role in the immune system helping to fight off infections. Deficiency of copper also has been associated with retained placenta, embryonic death, and decreased conception rates. It is a component of the enzyme ceruloplasmin, which is synthesized in the liver that assists in iron absorption and transport. Furthermore, Cu is an important part of superoxide dismutase, an enzyme that protects cells from the toxic effects of oxygen metabolites released during phagocytosis. Inadequate copper status may be related to an increased incidence of infections at calving, increased severity of infections, and a higher somatic cell count than that seen in copper-adequate cattle. Clinical signs of copper deficiency may include a discoloration of the hair coat from black to reddish). Adequate supplementations will help to avoid marginal deficiencies and support cattle to resist infections. Zinc is also an integral part of the immune system. Zinc is important for production of keratin, which lines the inside of the teat duct and helps to keep out micro-organisms that can cause mastitis. Zinc is an essential component of several enzymes involved in the synthesis of DNA and RNA, and has an antioxidant role by being part of a group of elements that induces the synthesis of metallothionein, which binds to free radicals.

β -Carotene and Vitamin A

The role of vitamin A and β-carotene in prevention of animal diseases is well documented. Vitamin A is necessary for all cellular division and differentiation and plays a key role in keratinization, and has an important role in maintaining epithelial tissue health and preserving the integrity of the mucosal surface. Beta-carotene, a precursor of vitamin A, functions as an antioxidant reducing superoxide formation within the phagocyte and can directly enhance immunity with reproductive and mammary benefits.

Feeding strategies for optimum health

The feeding management 30 days prepartum to 30 days post partum is critical for optimal milk yield, health and reproduction. A sound late pregnancy feeding programme is the key for improved lactation and reproductive performance of cow.

Dry period feeding

The pregnant cow in lactation, need rest for a certain period to allow reserve buildup and meet the forthcoming events of parturition. A dry period of 60 – 75 days is generally advised. The energy stored during this period can be mobilized to meet the deficit in early lactation. In addition to good quality green fodder, 2–3 kg concentrate is recommenced for reserve building. Proper feeding schedule should be adopted to achieve a body condition score of 3.5 in a 1 to 5 scale, during the late pregnancy period. Cows

that score 2.5 and less during early lactation will have lower fertility and health problems. At least 40 % of the total DM requirement (1% of cow's body weight) should be met through good quality green fodders and the mineral requirement should be met through additional inorganic/organic supplementation.

Transitional dry cow diets

This diet is designed to shift the cow from traditional dry cow diet (more fiber) to the early lactation diets (high in energy, protein, less fiber). Transitional diets should be fed for at least 3 wk before parturition, so that rumen microbes gradually get adapted to high-energy diets. The transitional dry cow diets should be formulated to minimize the incidence of metabolic disorders during lactation.

Early lactation diet

Soon after calving, it is not possible to meet the nutrient requirement through feeding alone due to reduction in appetite of cow. Hence, it is desirable to increase the grain portion as energy source and at least 20 % of the DM intake should be through good quality green fodders to maintain the rumen environment. After 12 week of calving the proportion of grain can be reduced as the cow can consume more of roughage to meet the energy demand. For meeting the energy requirement during early lactation certain additives like propylene glycol (70-100 g / day) can be added in the diet. Supplementation of niacin (3-5 g /day) will also help in improving the rumen environment and appetite of cow. In very high yielding cows feeding of bypass fat (100-200 g / day) will help in early return to positive energy status, thereby signaling the resumption of ovarian activity.

Mineral Supplementation

The most efficient method of providing supplemental minerals is through use of mineral supplements combined with concentrates, which assures an adequate intake of mineral elements by each animal as it consumes the nutrients. Indirect provision of minerals to grazing cattle includes use of mineral containing fertilizers, altering soil pH, and encouraging growth of specific pasture species. However this approach may not be always feasible due to the complex soil-plant-animal interrelationship. Direct administration of minerals to livestock in drinking water, mineral licks and mineral mixtures are also quite effective in preventing mineral deficiencies. In acute deficiency, drenches, slow releasing mineral boluses and injectable preparations are useful in correcting the disorder. But the most practical approach is to supplement through feeds and fodders which are rich sources of micronutrients.

Area-specific mineral mixture (ASMM)

Feeding of 'free - choice' mineral supplements could be the easiest way of supplementing minerals. Alternatively providing area - specific mineral mixture based on the deficiency of minerals in different agro-climatic zones is most appropriate and cost effective method of mineral supplementation. The former approach could sometimes lead to deleterious effect, as some of the minerals may be available in excess than requirements affecting utilization of other minerals. For example, excess of selenium affects sulphur utilization, excess of molybdenum and sulphur reduces copper absorption and excess of iron disturb copper metabolism. More practical method is of supplementing only the most deficient minerals through area specific mineral mixture by assessing the mineral content in soil, feeds and fodders and in animals in different agro-climatic zones. This approach has been found to improve the reproductive efficiency and health in crossbred cattle under field conditions and this technology has been successfully adopted.

Table1. Recommended nutrient content in ration of dairy animals

	Dry Cow		Lactating cow
	Traditional diet	**Transitional diet**	**Early lactation diet**
Days (pre partum)	21 - 60	< 21	-
Days (post partum)	-	-	< 30
DM intake (% B W)	2.0	2.2	2.5 – 2.7
CP (%)	14-15	16 - 18	19-21
UP (% of CP)	35 - 37	38 - 40	40-45
Energy (% TDN)	65	68	70-75
Fat (%)	2 – 2.5	3-4	4-5
Ca (%)	0.6	0.7	1.0
P (%)	0.26	0.3	0.36
Vitamin A (IU / Day)	22000	23000	24000

DM : Dry matter; BW : Body weight ; CP : Crude protein ; UP : Undegraded protein ; TDN : Total digestible nutrients ; Ca : Calcium ; P : Phosphorus

Table2. Prepartum nutrient status, potential metabolic disorders

S No.	Nutrient status		Possible disorder
	Deficiency	**Excess**	
1.	Energy	-	Ketosis, mastitis
2.	Ca, Mg	K	Milk fever, delayed uterine involution, mastitis
3.	Se, Vitamin E, Zn, Cu	-	Mastitis, Retention of placenta
4.	-	Na, K	Udder edema

Table3. Sources of micronutrients

Minerals	**Natural sources**
Calcium	Legumes, tree leaves, limestone, oyster shell
Phosphorus	Oil cakes, rice polish and rice/wheat bran
Sodium chloride, Potassium	Green fodders, Tree leaves, oil cakes, common salt,fish meal
Magnesium	Green fodder, oil cakes, legumes
Sulphur	Oil cakes, fish meal, meat meal
Copper	Green fodder, legumes
Zinc	Legumes, grasses, tree leaves
Iron	Green fodder, local grasses, legumes
Cobalt	Tree leaves, meat meal, fish meal
Iodine	Fish meal, protein supplements, iodised common salt
Selenium	Legumes, grasses, seleniferous plants
Manganese	Rice bran, alfalfa meal

Table 4. Major nutrient requirement of dairy cattle

Mature cow: last 3 months of gestation – Maintenance requirement						
Body weight	**DM (%BW)**	**CP (g)**	**TDN (kg)**	**Ca (g)**	**P (g)**	**Vitamin A (1000 I.U)**
400	2.1	480	4.2	14	14	21
450	2.1	514	4.6	15	15	23
500	2.0	546	5.0	15	15	24
550	2.0	579	5.3	16	16	26
600	1.9	629	5.7	17	17	27
Lactating cow: upto 12 weeks of lactation - Maintenance						
400	2.2	908	4.8	30	23	21
450	2.1	950	5.1	31	24	23
500	2.1	988	5.4	33	25	25
550	2.0	1028	5.7	34	26	27
600	2.0	1064	5.9	35	27	30
For each kg of milk production						
Fat (%)	**CP(g)**	**TDN(kg)**	**Ca(g)**	**P(g)**	**-**	**-**
3.5	71	0.31	2.6	1.9	-	-
4.0	79	0.31	2.7	2.0	-	-
4.5	86	0.33	2.8	2.1	-	-
5.0	93	0.35	2.9	2.2	-	-

Conclusions

Nutrition can influence the cow's resistance to mastitis. Mastitis control begins with implementation of the programme. Ensuring that the cow has adequate energy, minerals and vitamins for optimal milk production is essential for the maintenance of udder health and immune status. A holistic approach to mastitis control should be taken and nutritional management is one part of the control programme.

Following points should be considered to improve mammary health and control mastitis:

1. Follow a proper milking management practice which includes pre and post milking teat dipping.
2. Hygienic environment and proper dung disposal. Avoid animal lying on floor soon after milking. For this engage the animal for at least 30 minutes after milking by offering concentrate feed.
3. Supplement vitamins and trace mineral in diet to ensure immunity: Vitamin E- 1000 IU/ day for dry cow; 500 IU/ day for lactating cow, Selenium : 0.3 ppm in total diet, Copper : 10-15 ppm, Zinc : 40-60 ppm.
4. Use better bioavailable forms of micronutrients (Cu: Copper sulphate, Zn: Zinc sulphate, Se: Sodium selenate, chelated trace minerals).
5. Avoid metabolic disorders that can influence immune function and udder health.

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Dairy herd management practices for control of mastitis

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Dairy production system very widely around the world: species of predominantly buffalo and cattle, but large number of goats and sheep are also found for milk production. Herd size varies from single female animal to many thousand. Feeding system vary from extensive forage or grazing to total mixed rations. Animal may be fully pastured or fully housed. To ensure that this guide is totally applicable based on the principals that define better management practices. As a consequence their focus is necessarily the measure of success based on the outcomes for the animal rather than the provision of resources into the system. This guide identifies five key actions

- Stockmanship
- Feed and water
- Physical environment
- Husbandry practices
- Health management

The welfare of animal in dairy production systems can be assessed and monitored using a combination of measures that indicate the level of delivery within the five action areas.
These will be measures of the adequacy of the particular system in meeting the animal's needs. The selection of parameters to be used will therefore be specific to the dairying system under consideration. It includes elements of the following

- Observation of animal behaviour that indicates stress or distress. e.g.: incessant vocalization due to hunger or water deprivation , fighting because of mixed social groupings, dunging in the milking parlour or while being milked, kicking at the bucker or milking machine because of painful milking procedures, increased respiratory rate because of heat stress, fearfulness of humans because of ill treatment.
- Assessment of body condition
- Assessment locomotion score
- Assessment of relevant physiological indicators
- Assessment of environment stress such as excessive heat or cold, lack of shelter faecal accumulation and housing density
- Presence of lesions such as hock rubs, open sores or injuries
- Assessment of the level of training and skill of stockpersons and availability of veterinary assistance
- Assessment of the adequacy food and water resources to meet the needs of the animals
- Assessment of health management plans and records of animal treatments

Housing:

The effect of housing patterns on occurrence of subclinical and clinical mastitis was also investigated. The cattle sheds having asbestos sheets as roof in combination with stone slab flooring account 10.6 per cent followed by asbestos roof with concrete flooring as 8.47 per cent. In this study the trend in housing of animals indicated that there is highest availability (32.63 per cent) of cattle shed were with indigenous tiles on top as roof with stone flooring followed by thatch roof in combination with stone slab flooring as 19.06 per cent. The occurrence of subclinical mastitis was highest (14.19 per cent) in sheds with indigenous tiles on roof with flooring made up of stone slabs, followed by asbestos roof and concrete floor as 8.47 per cent. The incidence of subclinical mastitis was lowest (3.17 per cent) in sheds with tile roof in combination with katchha (muddy) floor. This clearly indicated that the occurrence of

subclinical mastitis in ordinary housing was controlled by sufficient hygiene practices by small or marginal farmers as they have comparatively small nos. of animals with enough personal care for the animals.

Parity:

The parity or no. of lactation by the lactating animals is very important phenomenon because it reflects the age and general health status of the animals. Accordingly, physiologically there will be changes in mammary health and productive performance. In our study, it was observed that the incidences of subclinical mastitis were recorded highest (25.75 per cent) in fourth parity followed by second (25.14 per cent) and third parity (23.95), respectively.

Seasons:

The occurrences of clinical and subclinical mastitis were observed highest in summer season followed by winter and rainy seasons, probably organisms causing mastitis were present at threshold level with less availability of fodder during winter season.

Detection of mastitis

In India, the diagnosis of clinical mastitis is straightforward. But sub clinical mastitis is more difficult to detect as the only evidence of disease is an abnormality of cell counts or other clinical pathogens. The cow appears healthy; the udder does not show any signs of inflammation and the milk seems to be normal. Sub clinical mastitis remains in the dormant state, but can develop at any stage into clinical mastitis, causing loss in milk production, and milk quality, and spreading infection to other cows in the herd. In subclinical mastitis, the problem lies with its detection. Once detected, it is useful to estimate the production loss associated with this invisible form of mastitis. The solution is some type of somatic cell counting. When the somatic cells are counted, subclinical mastitis is visualized and the milk production loss can be estimated. Thus somatic cell counting (SCC) is a very useful measure to increase the awareness of subclinical mastitis and its effect on production and milk quality. However in sub clinical mastitis, microorganisms and white blood cells (somatic cells) that fight infections are found in elevated numbers in milk.

Control of Sub Clinical Mastitis is more important than simply treating clinical cases because:

- The cows that have sub clinical mastitis are reservoirs of organisms that lead to infection for other cows.
- Most of the clinical cases start as sub clinical; thus, controlling subclinical mastitis is the best way to reduce the clinical cases.
- Changes in milk composition (reduction in calcium, Phosphorus, Protein and fat, and increases in sodium and chlorine) reduce its quality.

Sub clinical mastitis is not predicted by Physical examination of the udder or based on appearance of the milk, so various field based snapshot methods are followed .The sub clinical mastitis is a form of inflammatory reaction within the mammary gland is detectable only by indirect tests and cultural isolations. Detection of sub clinical mastitis requires repeated examination of milk particularly of incubated milk samples.

The tests Used for detection of mastitis:

1. Electrical Conductivity Meter Test
2. Somatic cell count (SCC)
3. California Mastitis Test:
4. SCC through Digital Reader
5. Ultrasonography

The prevention of mastitis can be achieved by adapting the following practices:

- ***Proper milk hygiene:***
  - Teats should be cleaned and dried before milking.

- If milk is filtered, the presence of particles in the filter indicates insufficient cleaning of teat during udder preparation or a lack of hygiene during attaching and removing of milking unit.

- ***Milking machine should function and be operated properly:***
  - Vacuum level in the milking unit should be between 275 and 300 mm of mercury.
  - Fluctuations may be reduced considerably.
  - The vacuum regulator should be kept clean and checked regularly for accuracy.
- ***Teat Dipping:***
  - Research indicates that the rate of new infection may be decreased by more than 50% when a suitable disinfectant is used.
  - Post milking teat dipping is most effective against *Staphylococcus aureus* and *Ster. agalactiae*, the two most contagious mastitis-causing bacteria.
  - Teat dipping does not affect existing infections.
- **Treatment of all quarters of all cows at drying off:**
  - The effective use of a long term antibiotic infused in each quarter of the udder at the last milking of lactation reduces the incidence of new infections during the dry period.
  - Dry cow therapy is the best way to cure chronic and subclinical mastitis that can rarely be treated successfully during lactation.
- **Culling chronically infected cows:**
  - Generally this method is effective because in most herds, only 6 to 8% of the cows account for 40 to 50% of all clinical mastitis.
- **Other practices:**
  - Deficiencies of selenium and Vitamin E in the diet have been associated with an increased rate of new infection.
- **Other practical management practices:**
  - Feed the cows immediately after milking so that they remain standing for at least for one hour before they lie down.
  - Milk the infected cows using designated equipment/machine and clean them same with disinfectants
- **Timely and properly treatment of all clinical cases:**
  - Adequate therapy must be decided by veterinarian and the cow should be handled accordingly to avoid the risk of spreading the disease.

Development of package of practices for effective control of sub clinical mastitis – a field experience:

Training cum demonstration programmes on detection and control of subclinical mastitis in lactating cows by using electrical conductivity meter, CMT kit and digital somatic cell counter were arranged for the benefit of the dairy farmers of Shivajinagar, Koramangla, Adugodi and Dhoddanekkundi areas *etc.* under Bangalore urban. Farmers in the areas under Kumbalgarh village and two adopted villages *viz*., Srirammnahalli, Rajanukunte and Honnenahalli in Doddaballapur taluk under Bangalore rural district were also trained. In these areas women dairy farmers have shown special interest.

Similarly demonstration cum training programmes were also arranged at Chkkaballapur and Boppanahalli villages of Mallur taluk under Kolar Milk Union, Kolar dist Kolar districts in which farmers have shown keen interest to learn and perfor the traing programme. Similar training programme was also arranged at NDRI Cattle Yard where farmers belonging to Andhra Pradesh, Karnataka and Kerala attended. Our efforts resulted in awareness creation about subclinical mastitis. Hence, they have started to implement the detection and control of subclinical mastitis. More than 650 farmers were provided *on-farm* and *off-farm* training and they have practiced at their own for further use at their herds.

After our demonstration, Karnataka Milk Federation (KMF) both in Bangalore and Kolar districts have initiated steps to control subclinical mastitis at their collection centres. This clearly indicates that the possibility of using techniques for qualitative analysis of milk immediately after milking as well as from bulk milk cooler becomes more useful. So, the efforts made by the NDRI SRS team helped in quality

improvement of milk production in the region.

Awareness about subclinical mastitis was also created among dairy farmers and entrepreneurs during National Krishi Mela/Exhibitions etc. organized by UAS, Bangalore at GKVK, Bangalore. Nearly 1000 dairy farmers were benefitted out of this demonstration.

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Quality milk production with emphasis on HACCP

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Nowadays quality consciousness is increasing amongst consumers. When it comes to consumption of milk and milk products, the concerns about quality increases many fold. To achieve highest level of milk quality major stakeholders, dairy farmers and Veterinarians and dairy industry *etc*. must play proactive role.

Maintenance of hygienic housing:

Maintenance of adequate hygienic conditions is very much necessary in and around the sheds, milking buyers etc. to minimize the infection. The dung should be removed immediately and proper drainage for the dung, urine, and waste water is necessary to keep the surrounding clean. The manure pit should be established in safe distance to prevent flies and insects entering into the sheds. Before milking initiates, the sheds should be kept clean and dry. After milking the sheds should be cleaned thoroughly, and dried and disinfected.

Proper Milking:

For clean milk production and to avoid intra mammary infection, application of proper milking procedure is important. In any organized dairy farm, milking is practiced through either hand milking or machine milking. In hand milking method, milking should be done with full hand method because it exerts an equal pressure on large teats of cows and buffaloes as well as it simulates the natural suckling process by calf.

Milking schedule:

Milking should be done at regular interval preferably for twice a day or thrice in a day if necessary. It is desirable to carry out the milking by the same milker to avoid stress to the cow. Milking order is preferred to implement i.e. freshly calved animals followed by other lactating animals in chronological order of calving and based on somatic cell counts. The infected cows are to be considered in different approaches possibly in quarantine sheds to minimize the spread of infection to other cows. During milking, abrupt change in timing, physical torture like beating of animals should be avoided to keep the milking environment very congenial to the lactating cows.

Cleaning of the animals:

Prior to milking, the animals are to be cleaned thoroughly to remove the any dirt/dust material and dung particles sticking on the thighs and udders. Proper cleaning of teats and udders prior to milking either by hand or by machine milking is an important task because of prevailing hygienic conditions surrounding teat canal there will be least chance of environmental pathogens to invade into the mammary gland. Potassium permanganate ($KMnO_4$) solution can be used to clean the udders. The animals should be kept quiet during the milking process. A soft music can be played in the sheds to keep the atmosphere serene and quiet. Further the milking should be practiced at fixed hours every day so that the animals get accustomed for milking at that time. Feeding concentrates at the time of feeding also helps to make the animals quiet and cooperate with the milking people.

Teat End Condition Scoring

Maintenance of healthy teat skin and teat-ends is a key part of any effective control of mastitis in a dairy herd. Changes to teat tissue, particularly the skin of the barrel, teat-end, and teat canal, alter the risk

of new intra mammary infection, either in the form sub-clinical or clinical mastitis based on CMT scores. Instruments and measurement techniques used to assess the condition of teat tissue include changes in teat thickness with a modified cutimeter, ultrasonography, sub-cutaneous oxygen tension, and pulse oximetry.

Teat condition used to assess the effects of milking management, milking equipment or environment on teat tissue and the risk of new intramammary infection. Veterinarians and others require a simple and reliable method for evaluating teat health in dairy herds.The relative influence of non-infectious factors affecting short, medium or longer term changes in teat condition are briefly discussed.

- Mainly short-term (single milking) effects include changes in color, swelling and firmness of the teat-end and barrel, and degree of openness of the external teat orifice.
- Medium-term effects (ranging from a few days to weeks) include changes in teat skin condition and incidence of petechial haemorrhages.
- Changes in teat-end hyperkeratosis appear to be longer-term effects (typically 2-8 weeks) in the absence of unusually harsh weather conditions.
- Very long-term effects (typically occurring over a few or many months) such as changes in size, tissue fibrosis and thickness of teats.

Cleanliness of the milking personnel:

To maintain the proper hygienic condition at the herd, the milkers should maintain cleanliness as it is practiced in animals. Their hands are to be washed with an antiseptic solution and kept dry. They should cut their nails properly to prevent any injuries to the teats. They also should wear a cap to prevent hair falling into the milk. They should be medically tested declared fit and free from any infectious diseases before engaging in milking duties so that cows cannot get zoonotic infection from humans.

Cleanliness of milking pails:

The milking pails used for milking operation should be cleaned thoroughly with clean warm water and with recommended detergent. After this step of washing, utensils are once again thoroughly washed with plain clean water and dried by keeping upside down. The lids should be closed properly and kept ready for the next milking. While transferring the milk into the transport cans, the milk should be filtered through a clean cloth to remove any particles. After milking operation, the pails are to be kept in a cool place and should not be exposed to direct sunlight. If the milk is to be transported to long distance it is cooled in a refrigerated room to prevent spoilage.

Storage and transport of milk:

During milking, milk will be at the body temperature. However, preserving the milk at that temperature is not possible because it gets deteriorated quickly due to its perishable nature. Hence, the milk should be chilled and stored properly at 4 - 5 ºC. Chilling of milk enhances the shelf life. While transporting milk to the dairy plants, the continuous cold chain should be maintained for maintaining the quality of milk.

HACCP Principles

The development and introduction of Hazard Analysis and Critical Control Points (HACCP) system at dairy farm levels is an important step towards ensuing quality milk production. ISO 22000 integrates the following HACCP 7 principles developed by the Codex Alimentarius Commission and dynamically combines them with PRPs (Prerequisite Programme) necessary to control and reduce any food safety hazards including milk identified for the end products delivered to the next step in the food chain to acceptable levels.

HACCP principles:

- Conduct a hazard analysis
- Determine the critical control points (CCPs)
- Established critical limit(s)
- Established a system to monitor control of the CCP

- Established the corrective action to be taken when monitoring indicates that a particular CCP is not under control
- Established procedures for verification to confirm that the HACCP system is working effectively and
- Established documentation concerning all procedure and records appropriate to these principles and their applications.

Models of the ISO 22000

The ISO 22000 model is a continuous improvement process based Food Safety Management System (FSMS) with systematic approach to developed, plan validate, establishe, implement, monitor, verify and improve the FSMS.

The production of clean milk involves thorough cleanliness at all stages of operation.

- Maintenance a healthy herd involves routine examination of cattle to ensure that they are free from any disease. Otherwise, this can spread infection to human beings through zoonotic disease which is transmittable *via* milk.
- Animal housing including milking area must be free from flies, rodents, vermin, dust, smoking, all manure dung and dust particles.
- Cow stalls must be cleaned with disinfectants consisting of quaternary ammonium compounds diluted with only 60% water for sanitizing purpose.
- Balanced feed which is free of pesticides is preferred to offer for animals. Ingredients of feed must be stored in moisture free conditions to inhibit the production of mycotoxin
- Utensils used for milking should be made up of aluminum or galvanized iron.
- The vessels are to be cleaned properly and sundry is to be done before and after milking.
- Chemicals like sodium hypochlorite in liquid form or iodophore, quaternary ammonium compounds, detergents like Teepol are also used for cleaning vessels. Ash or mud are not recommended for cleansing the utensils.
- Udder and teats of the cow are to be washed with warm water adding a pinch of potassium permanganate or sodium hypochlorite before and after milking. After washing the udder should be dried.
- Two or three drops of milk as first milk from each quarter are to be stripped to reduce bacterial count.
- It is to be ensured that the milker is free from any infection or wound and the person should keep proper personal hygiene. Wearing hand gloves by milker is preferred for quality milk production.
- Teats are to be dipped with iodophore solution after milking to prevent mastitis.
- The milk has to be cooled to a temperature below 5ºC by using household refrigerators / water coolers/ bulk milk coolers preferably within 2 hours after milking.

Conclusions

Concerted efforts are required on the part of the entire key stakeholder to ensure that from producer to consumer milk retains its quality in totality.

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Decision Support System (DSS) in Mastitis Management

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The safety of food of animal origin (milk, meat and egg) has received significant public media attention in recent past. Because of increase in the number of diseases transmitted form animal to human like avian influenza, *Escherichia coli* O157 infection, brucellosis, BSE etc. In many of these instances the control of the disease was by slaughter, but this kind of approach to disease management has raised questions about the legitimacy, ethics and long term future of intensive farming practices. To address the outbreaks of economically important livestock diseases in a population in quick and efficient manner it is essential that animal health authorities have access to appropriate information system which will serve as a guide in making decision. The details of animal health information will allow disease to be described in terms of the established epidemiological triad of individual, place and time. Individual animal-level analyses include estimates of the number of cases per head of population and for various subsections of the population (e.g. animals of a given age, sex, breed or type). Spatial analyses provide insight into geographical factors influencing the distribution of disease (e.g. proximity to pollutants, farming practices characteristic of a given area). Temporal analyses provide insight into short and long term variations in disease frequency. All three categories of analysis are useful in that firstly they provide an objectively measured point of comparison once control measures have been implemented and secondly, they provide information that can be used for hypothesis generation about factors associated with, or causing disease. Collection of additional information about the environment in which animals are located and events they are exposed to over their lifetime allows these hypotheses to be tested which in turn allow authorities to identify risk factors for disease. The surveillance and control of diseases like mastitis in farm animals with identified risk factors requires use of resources more effectively on target population is greatest need of attention. In the present day digital world the decision support system (DSS), which is a computer-based interactive software-based information system intended to help decision makers compile useful information from a combination of raw data, documents, and personal knowledge, or disease models to identify and solve problems and make decisions support at different levels (management, operations, and planning) of decision making activities.

There are two general classes of expert systems, knowledge-driven and data-driven. The difference between these classes is their method of operation. Knowledge-driven expert systems operate by collecting data to support or refute the inherent knowledge. These systems generally require substantial data collection and entry by the user. Conversely, a data driven expert system operates by applying data to a knowledge base, and conclusions are derived through deduction from that data. A properly designed DSS may serve as frontier technology for the animal health management, ultimately reducing the cost of production and in doing so helping alleviate one of the important obstacles to more widespread uptake of the technology itself.

An animal health DSS should have two main goals. The first is to provide authorities with the ability to trace animals from 'farm to fork', an essential requirement for food safety and documenting health status for domestic and international trading partners. The second is to provide a means to detect the emergence and re-emergence of diseases, allowing appropriate deployment of field operations and resources to deal with identified problems if and when they occur. Such a system promotes transparency in the state of animal health, allowing animal health policies to be based on the best available evidence. A typical animal health DSS work flow is depicted in figure 1 (Sanson, 1993)

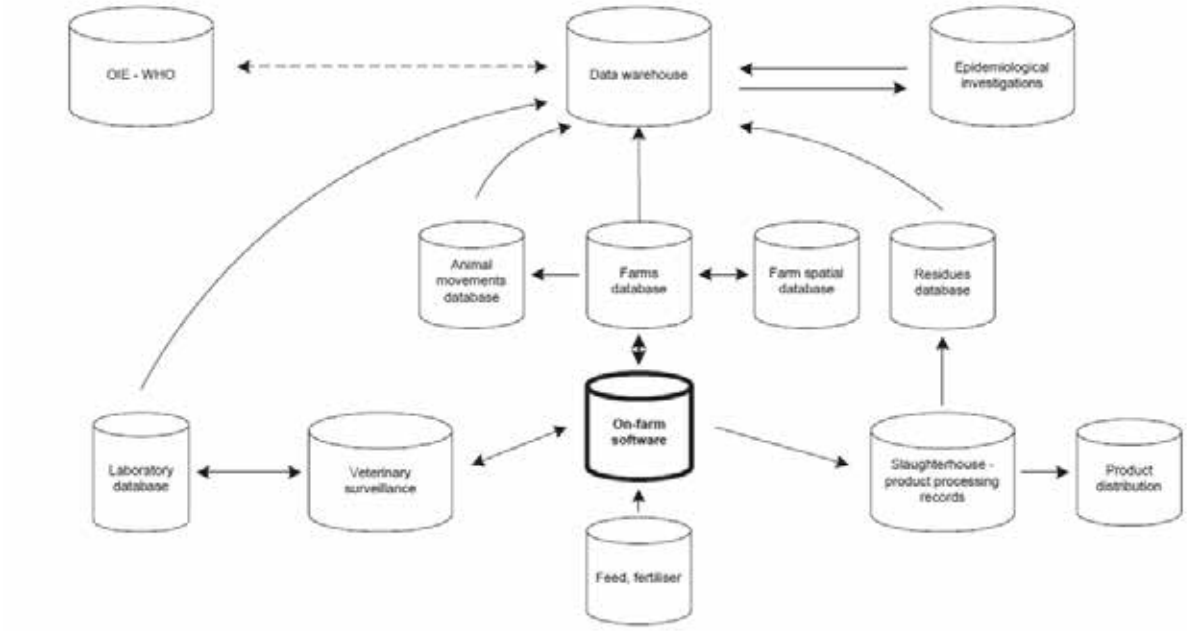

Fig 1: Schematic diagram representing the decision support system

Animal health DSS systems

1. NADRES (National Animal Disease Referral Expert System)
2. EpiMAN
3. MAST- Mastitis
4. RADAR (Rapid Analysis and Detection of Animal-Related Risks)
5. KODAVET/ISVET (*Koordiniertes Datenverwaltungs und Analysesystem des Veterinärdienstes Schweiz*)
6. DAFF (The National Livestock Identification System, Australia)
7. SENASA (The Sanitary Management System database, maintained by the National Service for Agrifood Health and Quality, Argentina)
8. AgriBase (New Zealand)
9. SISBOV (*Serviço de Rastreabilidade da Cadeia Produtiva de Bovinos e Bubalinos*, Brazil)
10. PDSR (Participatory Disease Surveillance and Response)
11. TADinfo (Transboundary Animal Disease Information System, FAO)
12. SAC (Animal Health Decision Support System, Scotland Agricultural College)

Decision support system in Mastitis

Mastitis is one of the most frequent and costly diseases in dairy cows (Halsa et al., 2007). The milk-recording data can be a useful source for information to support dairy farm management and control activities, both at the operational (short-term) and tactical (medium-term) levels of decision making. At the operational level, results from the most recent test day can be used to monitor current performance. At the tactical level, milk-recording data collected (e.g., over the past year) can be used to analyze the performance of the cows averaged for the entire year or as a function of month of year or even stage of lactation. Several computerized information systems have been developed to support the analysis of milk recording data. These include, at the operational level, a decision-support system for evaluating mastitis information, fuzzy-set based tools to monitor group-average milk yield and persistency values, and a prototype decision-support system for dairy cattle culling deployed over the Internet. One of its first kind of data-driven decision support system, MAST, was developed to summarize systematically the dairy

herd improvement (DHI) data related to mastitis. MAST determines weaknesses and problems of a mastitis control strategy by pinpointing problem areas, highlighting the scope of the mastitis problem, providing reference values for comparison, offering potential solutions to problem areas, and monitoring changes in the control strategy (Allore et al., 1995). In addition, several expert systems have been developed to support tactical level dairy management related to reproduction and covering milk production, nutrition, reproduction, and health. Detecting clinical mastitis (CM) is important to maintain an acceptable level of milk quality, to initiate an antibiotic treatment when necessary, and to safeguard the welfare of a dairy herd. When using automatic milking systems (AMS), farmers are not present during the milking process to check the cow and the milk visually for CM. Instead, they detect cases of CM by means of several information sources. The mastitis alert list, noting those cows and quarters that are likely to have CM, is one of these information sources. These lists are the output of a CM detection model available at the AMS. As input for such a detection model, the AMS uses in-line sensor information, with electrical conductivity being the trait most commonly measured in-line. Although farmers who use AMS are able to manage udder health sufficiently, udder health is under pressure on farms with AMS. One way to improve udder health on farms with an AMS is to improve the sensitivity (Se) of the currently available mastitis detection models. In addition, specificity (Sp) levels need improvement, as farmers are eager to minimize the additional labor of checking large numbers of false positive alerts, even at the expense of missing some true cases of CM. A level of 99% for the Sp and a minimum level of 70% for the Se have been suggested as goal for CM detection models when applied in practice.

Several models to detect mastitis using sensor information have been developed in the past and some studies have reported high levels of Se and Sp. However, few of these models are implemented in a practical AMS setting. There are several reasons why these models may not be applied in practice, such as the wide time windows used in some studies and the fact that some models were trained and validated using only clear cases of healthy and mastitic quarters or cows. Some of the studies developing a mastitis detection model based on electrical conductivity information used very long time windows; for example, de Mol et al. (1997) used a time window of 10 days before the actual CM case until 7 days after. Using wide time windows will result in models showing a good detection performance, but farmers need a CM alert within a very limited period before or only at the milking when CM occurs. Friggens et al. (2007) developed and validated their CM detection models with highly selected data including only those cows that clearly had CM and those that were clearly healthy. Such validation, however, will overestimate the detection performance of the model because field data include data from cows or (quarter) milkings that have a less clear mastitis status as well. A CM detection model should be able to deal with this so-called gray area.

One of the major problems of developing a CM detection model using sensor data is that the data are often noisy and incomplete. In addition, the low prevalence of CM results in highly imbalanced data, which makes modeling even more difficult. These problems make it worthwhile to look at tools other than the traditionally used statistical approaches to develop a detection model. One of these potential tools is data mining, which is the process of finding new and potentially useful knowledge from existing large databases. Decision-tree induction is a commonly used data mining technique that is often used for classification problems. Kamphuis et al. (2010a) reported a simple decision tree that was capable of detecting CM with a similar performance compared with detection models currently used by AMS. They suggested improving detection performance by using more CM cases and using quarter milkings (QM) that clearly had CM or were healthy in the training process. Also, combining decision-tree induction with bagging and boosting techniques was expected to improve detection performance.

In conclusion the outputs from DSS systems can be used to manage and control outbreaks of infectious disease in animals, identify factors associated with the presence of disease, provide an objectively measured point of comparison once control measures have been implemented and provide an additional means to detect emerging disease syndromes.

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Recent advances in Bovine Mastitis Treatment

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Bovine mastitis comprises of an inflammation of the mammary gland, which is almost always linked with bacterial infection. This seems to have emerged as an economically devastating disease hampering desired progress in dairy front. It affects every dairy operation and upto 50% of all dairy cattle according to National Animal Health Monitoring System (NAHMS) study, USA.

This review gives a brief overview of the important infectious agents with special reference to usage and activity of important antimicrobial drugs, penetration and tissue distribution in the mammary compartment, penetration into cells and its activity in the target area.

A total of about 140 microbial species, subspecies and serovars have been isolated from the bovine mammary gland. The pathogens have been broadly classified as causes of contagious, teat skin opportunistic or environmental mastitis.

- **Contagious mastitis pathogens**
  - *Staphylococcus aureus,*
  - *Streptococcus agalactiae*
  - *Mycoplasma bovis*
  - *Corynebacterium bovis*
- **Teat skin opportunistic mastitis pathogens**
  - Coagulase-negative *Staphylococci*
- **Environmental mastitis pathogens**
  - *Escherichia coli*
  - *Klebsiella spp.*
  - *Enterobacter spp*
  - *Arcanobacterium* (formerly *Actinomyces*) *pyogenes*
  - *Streptococcus uberis*
  - *Streptococcus dysgalactiae*
  - *Streptococcus equinus* (formerly referred to as *Streptococcus bovis*)
- **Uncommon mastitis pathogens**
  - *Nocardia spp, • Pasteurella, • Mycobacterium bovis, • Bacillus cereus*
  - *Pseudomonas spp, • Serratia marcescens, • Citrobacter spp,* • Anaerobic bacterial species*,* • *Fungi* and yeasts

Pathogenesis

Occurs as three stages: Invasion, infection and inflammation

- Invasion is the stage at which pathogens move from the teat end to the milk inside the teat canal.
- Infection is the stage in which the pathogens multiply rapidly and invade the mammary tissue. Multiplication of certain organisms may result in the release of endotoxins, as in coliform mastitis, which causes profound systemic effects with minimal inflammatory effects.
- Inflammation follows infection with varying degrees of clinical abnormalities of the udder and variable systemic effects from mild to peracute. Gross and subclinical abnormalities of the milk appear.

Clinical signs

- Gross abnormalities in milk (discoloration, clots, flakes, pus)
- Physical abnormalities of udder: acute - diffuse swelling, warmth, pain, gangrene in severe cases; chronic - local fibrosis and atrophy.
- Systemic response: may be normal or mild, moderate, acute, peracute with varying degrees of anorexia, toxemia, dehydration, fever, tachycardia, ruminal stasis, recumbency and death.

Characteristics of the different types of mastitis

Types of Mastitis	Characteristic sign or definition
Peracute	Swollen, red, painful quarter. Milk passes with difficulty. Fever over 41˚C. Cow has no appetite, shivers and loses weight quickly. Lactation often stops. Signs of toxemia.
Acute	Inflammation of the teat, fever above 39˚C, weak and dejected animal, lack of appetite. Drastic reduction in milk yield. Often follows calving and, less seriously, after cow goes dry.
Subacute	No apparent change in udder, presence of flaky particles in milk, especially in initial ejection. Subject appears healthy.
Subclinical	No clinical manifestations. 15 to 40 cases of subclinical for every clinical case. Milk appears normal. Only change is detection of pathogenic agent in analysis and increased somatic cell count. Mostly caused by *Staphylococcus aureus*.
Chronic	Repeated but mild clinical attacks, generally without fever. Lumpy milk, quarters sometimes swollen. Quarter may become hard (fibrous indurations). Antibiotic treatments often do not work.
Gangrenous	Affected quarter is blue and cold to the touch. Progressive discolouration from the tip to the top. Necrotic parts drop off. Cow often dies.

Diagnosis

- Detection at the herd level: Bulk tank milk somatic cell count (SCC). Culture of bulk tank milk
- Detection at the individual cow level: abnormal looking milk, culture of composite or quarter milk samples.
- Indirect tests include SCC of composite or quarter milk samples, California Mastitis Test (CMT) of quarter milk samples, inline milk conductivity tests of quarter milk samples
- Use of selective media to differentiate Gram-positive and Gram-negative pathogens in cases of clinical mastitis

Treatment

In India, subclinical mastitis is found more prevalent (varying from 10-50% in cows and 5-20% in buffaloes) than clinical mastitis (1-10%). Effective and economical mastitis control programs rely on prevention rather than treatment. Antimicrobials (ATMs) have been used as a mainstay to treat mastitis for more than fifty years, but consensus about the most efficient, safe, and economical treatment is still lacking. Dua (2001) reported the loss due to mastitis in India was about ₹ 6053.21 crores annually.

Treatment of clinical mastitis in lactating cow

1. Mild cases of clinical mastitis (abnormal secretion only) may not require treatment; however, all clinical mastitis episodes accompanied by an abnormal gland or systemic signs of illness should be treated with antimicrobial agents given by intramammary infusion (all cases) and parenterally (selected cases).
2. Acute and peracute mastitis cases also require supportive therapy (fluid and electrolytes) and nonsteroidal anti-inflammatory agents (NSAIDs).

Antimicrobial therapy

The bovine mammary gland is a difficult target for antimicrobial treatment. Penetration of substances into milk when administered parenterally or absorption and distribution throughout the udder when infused intramammary (IMM) depends on their pharmacokinetic characteristics. The extent to which a drug has access into milk when given systemically, or is absorbed and distributes throughout the udder when given intramammary (IMM), depends on the following properties.

- Lipid solubility
- Degree of ionization
- Extent of binding to serum and udder protein
- High lipid solubility, poor degree of ionization and less plasma protein binding contributes to a better transfer into milk. With IMM preparations, the type of vehicle is also important.

Antimicrobial activity

The knowledge about the pharmacological aspects of the antimicrobials is essential before choosing the drug in the treatment of mastitis. Antimicrobials exhibit three major patterns of activity.

1. First pattern is characterized by concentration dependent killing. Higher concentrations would kill organisms more rapidly and more extensively than lower levels. This pattern is observed with aminoglycosides and fluoroquinolones**.** Increasing concentrations at the site of infection increase the bacterial kill rate.
2. The second pattern is characterized by time-dependent killing and minimal to moderate persistent effects. Exceeding the minimum inhibitory concentration at the site of infection for a prolonged percentage of the interdosing interval correlates with improved efficacy. This pattern is observed with β-lactams, macrolides, and lincosamides.
3. The third pattern is also characterized by time-dependent killing, but the duration of the persistent effects is much prolonged. This can prevent any re-growth during the dosing interval. This pattern is observed with azithromycin and tetracyclines.

Pharmacokinetic considerations

The mammary gland is a rather immunologically weak organ; the phagocytic activity in the mammary gland is poor. In general, antimastitic drugs should preferably have bactericidal action, as phagocytes act normally immediately after milking, but as time elapses they incorporate fat globules becoming "engorged" thus diminishing its phagocytic capacity. As a consequence, milk phagocytes are less effective than plasma ones. Milk should not interfere with antimicrobial activity.

i. Parenteral antimicrobial treatment

The effect of the antimicrobial drug depends largely on the drug characteristics, the dose, the bioavailability of the molecule, the ability to penetrate the mammary gland and the microorganism susceptibility.

An ideal antimicrobial for systemic therapy of mastitis should have the following properties:

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

ii. Intramammary antimicrobial treatment (IMM)

The choice of antimicrobial agents for intramammary infusion should be based on:

- Spectrum of bacteria controlled
- Diffusibility through mammary tissue
- Cost

Table 1 -Target of antimicrobial therapy in clinical mastitis due to different pathogens

Mastitic pathogen	Milk ducts	Udder tissue	Cow (System)
Streptococcus agalactiae	+ + +	-------	-----
Other *Streptococci*	+ + +	+	---
Staphylococcus aureus	+	+ + +	---
Coagulase-negative *Staphylococci*	+ + +	---	---
Arcanobacterium pyogenes (summer mastitis)	---	+ +	+ + +
Coliforms	+	--	+ + +

After administration of an IMM infusion, the contact between the antimicrobial agent and the pathogen within the mammary gland is subjected to a series of successive events.

1. **Pharmaceutical phase:** This begins after drug administration and includes the following steps • Disintegration of the formulation • Drug dissolution • Liberation of the drug in milk
2. **Pharmacokinetic phase:** Assumes the presence of the drug in milk (drug availability) and includes the following events;

• Absorption (milk**:** plasma) • Distribution (local and systemic) • Metabolism (systemic) • Excretion (local and systemic).

3. **Pharmacodynamic phase:** The effect of drug against bacteria at the infection site. The frequency of administration for intramammary formulations is dependent primarily on the milking schedule, as the antibacterial agents are primarily cleared by milk removal. The target site may depend on the causative agent: *Streptococci* are known to remain in the milk compartment, but *S. aureus* penetrates udder tissue and causes deep infection. Efficacy of IMM treatment varies according to the pathogen, with the **best therapeutic response** being shown for mastitis caused by ***Streptococci*, coagulase-negative *Staphylococci*, and *Corynebacterium spp*.**

Benefits

1. The benefits of IMM administration are the high concentrations reached in milk and less loss due to drug absorption and transfer processes through biological membranes as the drug is directly infused into the diseased quarter. For example, concentration of penicillin G in milk after IMM administration is 100-1000 times as high as the concentration after systemic (parenteral) administration.
2. Intramammary antibiotic therapy is appropriate for Streptococcal mastitis. The cure rate of ***S. agalactiae*** using intramammary infusions in lactating cows exceeds 95%.

Disadvantages

1. Uneven distribution of various compounds within the udder
2. Risk of mammary contamination by bacteria inoculation through the teat canal and the possible irritation of udder tissue by the formulation
3. *S. aureus* mastitis cannot be completely cured only by this route.

Treatment of *Staphylococcus aureus* mastitis

***S. aureus* mastitis is refractory to most of the antimicrobials for the following reasons:**

- Adaptive response of the pathogen to survive in the mammary gland despite the presence of antibiotics and the inability of host defenses to clear the pathogen.

- Infectious bacteria in chronic mastitis may survive intracellularly at low pH and remain quiescent and protected from the action of antibacterials and host defenses.
- Re-infections occur due to newly acquired strains or from persistence of the original infective organism
- Small-colony variants (SCV) phenotype of *S. aureus contributes* to chronic mastitis.
- Ability of the isolate to produce *ß*-lactamase producing persistent infection with frequent relapse leading to chronic disease.
- Formation of biofilm and L-forms.

Table 2 - Classification of antibacterial drugs according to their potential distribution throughout the udder after parenteral and intramammary administration

TYPE OF DISTRIBUTION	PARENTERAL	INTRAMAMMARY
Good distribution	Quinolones	Quinolones
	Sulphanilamide	Nitrofurans
	Erythromycin	Sulphanilamide
	Oleandomycin	Dapsone
	Tylosin	Erythromicin
	Spiramycin	Oleandomicin
	Lincomycin	Tylosin
	Clindamycin	Spiramycin
	Penethamate	Lincomycin
	Trimethoprim	Clindamycin
	Tiamulin	Penethamate
		Ampicillin
		Amoxicillin
		Hetacillin
		Cephalexin
		Trimethoprim
		Novobiocin
		Rifamycin
Limited Distribution	Penicillin G	Penicillin G
	Cloxacillin	Cloxacillin
	Ampicillin	Oxacillin
	Amoxicillin	Cephaxazole
	Cephalosporins	Cephalonium
	Tetracycline	Cephapirin
	Novobiocin	Cephacetril
	Rifamycin	Tetracycline
	Fucidic acid	
Poor distribution	Dihydrostreptomycin	Bacitracin
	Neomycin	Dihydrostreptomycin
	Kanamycin	Neomycin
	Aminocidine	Kanamycin
	Spectinomycin	Aminocidine
	Gentamicin	Gentamicin
	Polymixin	Polymixin
	Vancomycin	

Current recommendations in the treatment of *S. aureus* mastitis

Although complete cure is difficult to achieve with ***S. aureus*** **mastitis**, 30 - 60% clinical cure rates was possible when the following intramammary infusions, given daily at 24-hour intervals.

1. Sodium cloxacillin (200-600 mg for three infusions)
2. Tetracyclines (400 mg)
3. Penicillin-streptomycin combination (100 000 units - 250 mg)
4. Penicillin-tylosin combination (100 000 units - 240 mg)
5. Novobiocin (250 mg per infusion for three infusions)
6. Cephalosporins - most strains of *S. aureus* are sensitive to cephapirin

Best results are achieved by combination of intramammary and parenteral antimicrobial treatment or use of extended intramammary treatment for 4-8 days.

Treatment of coliform mastitis

A combination of broadspectrum antimicrobial agents administered parenterally and by intramammary infusion, fluid and electrolyte therapy, frequent stripping out of the affected glands with the aid of oxytocin, and anti-inflammatory drugs have been used with varying degrees of success. *E. coli* isolated from the mammary glands of cattle are theoretically susceptible to third-generation cephalosporins (such as ceftiofur), fourth-generation cephalosporins (such as cefquinome), fluoroquinolones, gentamicin, amikacin, trimethoprim-sulfonamide and oxytetracycline.

Danofloxacin, a fluoroquinolone (FQ) antimicrobial drug developed for use in veterinary medicine shows a broad spectrum of activity in a single injection against most Gram-negative, Gram-positive bacteria and mycoplasma.

Dry cow therapy

Dry cow therapy is the use of intramammary antimicrobial therapy immediately after the last milking of lactation and is an important component of an effective mastitis control program. Intramammary infusion of long-acting antimicrobial agents at drying off provides the best treatment for subclinical mastitis due to contagious pathogens, decrease the number of existing infections and prevent new infections during the early weeks of the dry period. Dry cow therapy should be routinely administered and remains one of the cornerstones of an effective mastitis control program.

Blanket dry cow therapy: It is treatment of all four quarters at drying off.

Selective dry cow therapy: It is based on treatment of only those quarters that are infected.

Intramammary infusions approved for dry cow therapy contain high levels of antimicrobial agents in a slow-release base that maintains therapeutic levels in the dry udder for long periods of time to eliminate existing infections due to *S. aureus* and *S. agalactiae* at drying off. Cloxacillin, nafcillin, and cephalosporins are popular for this purpose; for example, a recommended treatment is cephapirin or sodium cloxacillin in a slow-release base with an expected cure rate of 80% against *Streptococci* and 60% against *S. aureus*. Most dry cow preparations maintain an adequate minimum concentration in the quarter for about 4 weeks, but some persist for 6 weeks.

New delivery systems for intramammmary infusions

Liposomes, microparticles and nanoparticles may be considered potential delivery systems in the treatment of bovine mastitis caused by *S. aureus* since they may be taken up by the phagocytes liberating the active once inside. Both types are spherical particles with an average diameter of between 0.05 μm and <5μm. Liposomes are phospholipidic particles with an aqueous core. Due to their amphiphilic structure, they can incorporate either lipophilic or hydrophilic compounds. Nanoparticles are polymeric particles which can be used in the effective delivery of antimicrobial agents.

Although lot of research and variety of antibacterials are in vogue in the treatment of bovine mastitis, the pattern of mastitis occurrence is significantly increasing in both cattle and buffaloes which is

a major challenge for field veterinarians and researchers. It is prudent that mastitis better be prevented than be treated / cured.

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Recent advances in diagnosis and treatment of Bovine Mastitis

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Introduction

Mastitis is inflammation of the parenchyma of the mammary gland regardless of the cause. Mastitis is characterized by physical and chemical changes in the milk and pathological changes in the glandular tissue. A large proportion of mastitis glands are detectable neither by manual palpation nor by visual examination of the milk. These types of mastitis come under the classification of subclinical mastitis. Diagnosis of subclinical mastitis depends largely on indirect tests, which depend, in turn, on the somatic cell concentration (SCC) or electrolyte (Sodium and Chloride) concentration of milk. Hence definition of mastitis can be "disease characterized by the presence of a significantly increased SCC in milk from affected glands". The increased SCC is due to an increased neutrophil concentration representing reaction to glandular tissue injury.

The incidence of clinical mastitis ranges from 10 -12 per cent per cows at risk per year. Prevalence of intramammary infection is about 50 per cent of cows and 10-25 per cent of quarters.

A total of about 140 microbial species, subspecies and serovars have been isolated from the bovine mammary glands. These pathogens have been further classified as causes of contagious mastitis, teat skin opportunistic mastitis and environmental mastitis. Contagious pathogens are transmitted at the time of milking. Teat skin opportunistic pathogens take any opportunity to induce mastitis whereas environmental pathogens are from the environment and induce mastitis between milking.

Clinically mastitis is characterized by gross abnormalities in milk such as discoloration, clots, flakes and pus, physical abnormalities of udder including acute diffuse swelling, warmth, pain, gangrene in severe cases, chronic – local fibrosis and atrophy. Systemic response to mastitis may be normal or mild, moderate, acute, peracute with varying degrees of anorexia, toxemia, dehydration, fever, tachycardia, ruminal stasis, recumbency and death.

Diagnosis of mastitis

The initial diagnosis of clinical mastitis is made during the routine examination. Laboratory culturing of samples for bacteria and Mycoplasma and for determining the antimicrobial susceptibility of organism is the next step. Diagnosis of a clinical mastitis generally will not present any problem to the clinician.

Detection of sub-clinical mastitis is a real challenge to Veterinarian. Culturing large numbers of milk samples is expensive and impractical for field use. Hence much attention has been given the development of indirect test that predict the presence of an intramammary infection. Currently available indirect tests detect only the presence of inflammation but are of little value as screening tests. Laboratory examination is the only way that subclinical mastitis can be detected.

Detection of subclinical mastitis at the herd level

The prevalence of subclinical mastitis or intramammary infection is monitored by determining the bulk tank milk SCC and the most likely mastitis pathogens are identified by culturing bulk tank milk. These two methods are recommended to diagnose the presence and prevalence of mastitis on a herd basis.

The SCC of bulk tank milk is an indirect measure of the prevalence of mastitis within a dairy herd. The SCC is increased because of subclinical mastitis associated with Gram-Positive bacterial intramammary infections. There is a good correlation between the number of Streptococci (*S. agalactiae, S. dysagalactiae* and *S. uberis*) colony-forming units found in the bulk tank milk and its SCC. The number of colony-forming units of *S. aureus* is moderately correlated to the bulk tank milk SCC.

The SCC of bulk tank milk has become a widely used test because it provides a sensitive and specific indicator of udder health and milk quality. The sample for analysis is obtained by agitating the milk for 10 minutes and collecting a sample from the top of the bulk tank milk using a clean dipper. Milk processing plants in most developed countries use automatic electronic somatic cell counters routinely in order to provide a monthly report of the bulk tank milk SCC. The bulk tank milk SCC is extremely useful increasing awareness of the existence of a mastitis problem, so that when the SCC of bulk tank milk exceeds permissible limits further investigation of the herd is indicated. It is not possible to use the bulk tank milk SCC to determine the number of cows in a herd affected by mastitis but it is possible to estimate fairly accurately the number of affected quarters. A bulk tank milk SCC of more than 300,000 cells per mL is considered to indicate a level of mastitis in the herd that warrants examination of individual cows.

Culturing of bulk milk is a useful technique for screening for major mastitis pathogens. The culture of *S. aureus* and *S. agalactiae* from bulk tank milk is a reliable indicator of infection by those pathogens in the herd. The number of these pathogens found on culture is determined by the number of bacteria shed, the number of infected cows, the milk production level of infected cows relative to herd mates and the severity of the infection. A single culture of bulk tank milk has low sensitivity but high specificity for determining the presence of S.agalactiae or *S. aureus* in the herd. In general, the sensitivity of a single bulk tank milk culture to detect the presence of intramammary infections due to S.agalactiae ranges from 21-77 %, for *S.aureus* it ranges from 9-58% and for *M.bovis* it is 33%.

Detection of mastitis at the individual cow level

Abnormalities of the udder and gross abnormalities of milk in cattle with clinical mastitis assists in diagnosis of clinical mastitis in a cow. In individual cows with clinical mastitis, culture of the secretion from an infected quarter can be done. Individual cow mil can be cultured as part of herd examination for mastitis or on individual quarter samples or on composite samples including milk from all four quarters. However individual quarter samples are preferred because the costs of treatment dictate that the least possible number of quarters be treated. Milk sampling for culture must be carried out with due attention to cleanliness since sample contaminated during collection are worthless. The first two or three streams of milk are rejected because their cell and bacterial counts are likely to be a reflection of the disease situation within the teats rather than within the udder as a whole. The new few steams, the premising sample, is the approved one because of its greater accuracy. For complete accuracy a premilking and a post milking samples are taken. If tuberculosis is suspected, the last few streams are the critical ones. A milk sample is considered contaminated when more than three species of bacteria are isolated. A quarter is considered to be infected when the same bacteria is isolated in at least two out of three milk samples. A quarter is considered to be cured when bacteria, isolated at drying off, are not present in any samples 28 days after calving.

Indirect tests for subclincal mastitis

Indirect tests include SCC using automated electronic counters, the California Mastitis Test, increase in electrical conductivity of milk and increases in the activity of cell associated enzymes such as NAGase in milk. ELISA tests to detect neutrophil components have been developed but are not commercially available. Of these indirect tests, only the CMT and electrical conductivity can be used cow side, with CMT providing a more accurate screening test than electrical conductivity.

Healthy quarters have a SCC below 100,00 cells/mL and this cutpoint should be used to indicate the absence or presence of intramammary infection on a gland basis. This cutpoint looks very solid for a gland, because many milk components differ from normal values whenever the SCC exceeds 100,000 cells/mL. An exciting new development in mastitis diagnosis is the portable somatic cell counter which provides SCC information immediately.

The CMT is the most reliable and inexpensive cowside test for detecting subclinical mastitis. It is a modification of Whiteside test. The CMT reagent contains a detergent that reacts with DNA of cell nuclei and a pH indicator (bromcresol purple) that changes color when the milk pH is increased above the

normal value of approximately 6.6. Cows in the first week after calving or in the last stages of lactation may give a strong positive reaction.

The NAGase test

The NAGase test is based on the measurement of a cell-associated enzyme (N-acetyl beta D glucosaminidase) in the milk, a high enzyme activity indicating a high cell count. NAGase is an intracellular lysosomal enzyme derived primarlily from neutrophils but also from damaged epithelial cells. The test is suited to be rapid hanlding of large numbers of samples because of the ease of its automation, and the test can be done on fresh milk and read on the same day. The samples should be frozen and thawed before analysis to induce maximal NAGase activity. The NAGase test is reported to the most accurate of the indirect tests and as good as SCC in predicting the infected status of a quarter. The NAGase test uses a less sophisticated reading instrument than the average automatic cell counters.

Electrical Conductivity test

This test is based on the increase in the concentration of sodium and chloride ions and the consequent increase in electrical conductivity in mastitic milk. The electrolyte changes in milk are the first to occur in mastitis. Electrical conductivity is attractive as a test because it measures actual injury to the udder rather than the cow's response to the damage, as in SCC and NAGase test. This test also assists in early detection of mastitis especially mastitis due to *S. aureus* and *S. uberis.*

Bovine Serum albumin assay, Determination of antitrypsin activity, Modified Aulendofar mastitis Probe (MAMP) test, determination of Adenosine triphosphate activity are other diagnostic tests employed in diagnosis of mastitis in bovines.

Ultrasonographic test

Two-dimentional ultrasonograhic images of the gland cistern, parenchymal tissue and the teats are easily obtained using a 5, 7.5 or 8.5 MHz linear array transducer. Mastitis produces an increased heterogeneous echogenicity to the milk in the gland cistern, compared to an uninfected quarter.

Biopsy of mammary tissue

A biopsy of mammary tissue can be used for histological and biochemical evaluation in research studies. This methodology is not employed in diagnosis of mastitis in clinical set up.

Treatment of Mastitis

In the early part all forms of both clinical and subclinical bovine mastitis were treated with a wide variety of antimicrobial agents either by intramammary infusions or parenterally, commonly by both routes in acute and peracute cases.

The treatment strategy will depend on whether the mastitis is clinical or subcliical and the health status of the herd including its mastitis history. If treatment is indicated, the major decision is whether to administer antimicrobial agent parentally or by intramammary infection.

Veterinarian should always ask and answer four questions related to antimicrobial therapy in bovine mastitis.

- Is antimicrobial therapy indicated?
- Which route of administration should be used?
- Which antimicrobial agent should be administered?
- What should be the frequency and duration of treatment?

There is marked difference in the bacteriological cure rates of the various major mastitis pathogens after therapy during lactation. The outcome of treatment during lactation is poor for cases of *S. aureus* mastitis. On the other hand, *S. agalactiae* responds extremely well to lactation cow therapy and all

infected cows should be treated. Heat, pain and swelling of the affected quarter indicate the need for antimicrobial therapy.

The second decision is the route of administration. The goal of antimicrobial treatment is to attain and maintain an effective concentration of antimicrobial agent at the site of infection. In general, infections confined primarily to the milk and duct (*C. bovis*, coagulase negative Staphylococci) are easily treated with intramammary antibiotics. In contrast, infections due to mastitis pathogens with potential for systemic infection (*E. coli, K. pneumoniae, M. bovis*) are best treated with parenteral antibiotics. Mastitis pathogens that are the most difficult to treat are those that are principally infections of parenchymal tissue (*S. aureus, A. pyogenes*). This is because it is more difficult to attain and maintain an effective antibiotic concentration at this anatomical site when administering by intramammary or parenteral route.

The third decision is the antimicrobial agent. The selection of the antimicrobial class for the particular mastitis pathogens has traditionally been based on culture and susceptibility testing or previous experience. But the *in vivo* and *in vitro* results may vary due to various factors including composition of mastitis milk and pharmacokinetics.

The fourth decision to be taken up by Veterinarian is the frequency and duration of treatment. The frequency of administration for parenterally administered antimicrobial agent is dependent primarily on their pharmacokinetics and pharmacodynamics. Fluoroquinolones and aminoglycosides are concentration dependent antimicrobial agents where increasing concentrations at the site of infection increases the bacterial kill rate. Macrolides, beta-lactams and lincosamides are time-dependent antimicrobial agents where exceeding the minimum inhibitory concentration at the site of infection for a prolonged percentage of the interdosing intervals correlates with improved efficacy. In contrast, the frequency of administration for intramammary formulations is dependent primarily on the milking schedule, in that these agents are primarily cleared by milk removal.

Intramammary Antimicrobial Therapy

For reasons of convenience and efficiency, antimicrobial udder infusions are in common use for the treatment of certain causes of mastitis in lactating cows and for dry cow therapy. The cure rate of *S.agalactiae* using intramammary infusions in lactating cows exceeds 95%. Disposable tubes containing suitable antimicrobials in a water-soluble ointment base are ideal for dispensing and for the treatment of individual cows. The choice of antimicrobial agents for intramammary infusion should be based on spectrum of bacteria, diffusibility through mammary tissue and cost.

The advantage of this route are high concentrations of antimicrobials achieved in the milk compartment of the mammary gland and low consumption of the antimicrobial substances as the drugs are administered straight to the infection site. Disadvantages associated with route of administration of antimicrobial agent are uneven distribution, risk of contamination while infusing the drug and possible irritation of the mammary tissue due to infused antimicrobial agent.

Intramammary preparations with combinations of two or even three antibiotics are introduced in the market due to suggested synergistic action. The evidence of their efficacy against Coliform Mastitis is still lacking.

As a general rule, oily preparations can be administered once daily but there is disadvantage that medicine may not spread into deeper tissues. On the other hand, aqueous preparations are well absorbed, well distributed, spread deeper. But these preparations have short acting period and hence need to be repeated at least twice daily.

The dose of different antimicrobial agents for intramammary preparation (per quarter) is follows

Erythromycin 300 – 600 mg, Cloxacillin 500 mg, Ampicillin 250 -500 mg, Chlormphenicol 250 -300 mg, Gentamicin 75 – 100 mg, Amoxicillin 250 – 500 mg, Tylosin 1 g, Carbenicillin 5 g, Ceftrioxone 250 -500 mg, Cefazolin 150 -500 mg and Cefatoxime 250 mg.

Parenteral Antimicrobial Therapy

Parenteral antimicrobial therapy should be considered in all cases of mastitis in which is there is an abnormal gland or abnormal cow (fever, decreased appetite or inappetence). The systemic reaction can usually be brought under control by standard doses of antimicrobial agents but a bacteriological cure of the affected glands is seldom achieved because of relatively poor diffusion of the antimicrobial from the blood into the milk. The rate of diffusion of antimicrobial agents is greater in affected quarters than in normal quarters. Parenteral treatment is also recommended when the gland is markedly swollen and intramammary infusions are unlikely to diffuse to all parts of the glandular tissue. To achieve adequate therapeutic levels of an antimicrobial in the mammary gland by parenteral treatment it is necessary to use higher than normal dose rates daily for 3-5 days.

Intravenous administration of antimicrobial agents would in general produce higher concentration of agents in milk, but it is often unpractical in field conditions and IV administration is not possible in antimicrobial preparations in oily vehicles. The slowly absorbed antibiotic preparations for intramuscular use are the worst choice in mastitis because they do not generally produce therapeutic concentrations in milk or tissues. One more additional problem for the practitioner is that dosage recommendations of many antibiotic preparations for adult cattle are too low with regard to the MIC of the target bacteria. Repeated intramuscular injections of large volumes of antibiotics are not ideal from the animal welfare point of view.

There are very few antibiotics which, from both the PK and PD point of view, would be ideal for systemic mastitis treatment. Even if the drug has ideal characteristics in theory, the treatment results from the clinical trails may still be disappointing. Hence many a times there is complaint of poor response when antibiotics are administered parentrerally.

Anti-Inflammatory Agents

NSAIDs have been evaluated for the treatment of acute and per acute mastitis. NSAIDs have beneficial effects on decreasing the severity of clinical signs. NSAIDs appear to ameliorate systemic abnormalities to a greater degree than corticosteroids.

Supportive Therapy

Supportive therapy including the intravenous administration of large quantities of isotonic crystalloid fluids is indicated in cattle with severe systemic illness. Styptics are to be used wherever it is warranted. Frequent milking, immunoglobulin therapy, oxytocin therapy are other supportive therapy employed in treatment of bovine mastitis.

Adjunctive Therapy

Cytokines may be useful as adjunctive therapy with existing antimicrobials to improve therapeutic efficacy, particularly in lactating cows. Cytokines are natural regulators of the host defense system in response to infectious diseases.

Trisodium citrate given at the rate of 30 mg /kg body weight orally once a day for 10 days has been claimed to be effective in treatment of mastitis to the extent of 60-70%.

Permanent Drying Off Of Quarter

Chronically affected quarters which are incurable warrants permanent drying off of quarter. Permanent drying off can be brought about with chemicals such as 30 – 60 ml of 5% silver nitrate, 20 ml of 5% copper sulphate, 100 – 300 ml of 1in 500 acriflavin solution, 60 ml chlorhexidine. If a severe reaction is noticed on infusion of these chemicals, it is advisable to milk out very frequently till the symptoms subside. If no reaction is noticed, the chemical may be left to act for 7 – 14 days duration. Some cases may need second treatment but in majority of cases, one treatment is sufficient to dry off the quarter.

The control programme on mastitis revolves around two basic concepts.

1) Assessment of herd's mastitis status
2) Implementation of recommended mastitis control program.

Assessment of Herds Mastitis Status

This involves checking of mastitis at quarter level, cow level, and herd level. The herd level check is usually by means of bulk milk cell count, which is done at monthly intervals. A bulk milk cell count of above 300000 cells to 500000 cells per ml of milk is considered as threshold value to improve the mastitis control program. The cow level check is examination of the milk of the individual cows by using different tests like CMT, SCC, NAGase and electrical conductivity. The quarter level check involves collection and examination of milk from all four quarters of all cows, or individual cows which have been identified as infected by a cow level check.

The options in mastitis control are

1) Eradication
2) Limitation of the infection rate
3) Legislative control
4) Voluntary program.

Recommended mastitis control programme

The basic program suggested /followed in many countries are with the following objectives.

I. Reducing the duration of infection

This is achieved by detection of infected quarters, treatment of infected quarters during lactation and dry period and culling of chronic cases.

II. Reducing the new infection rate

This revolves around milking hygiene especially disinfection and drying teats before applying milking machine cups, dipping all teats in efficient teat dips after each milking disinfecting or back flushing cups after each milking, adequately servicing and maintaining milking machinery etc, other factors which have bearing on infection rate are milking order, drying off, nutrition, vaccination, housing, fly control tail amputation etc.

III. Monitoring the infection rate

This is very important in assessing the control programme. Good managemental practices in housing, use of teat dips, disinfectants, vaccines and administration of compounds like Vit E, Selenium have been reported to be effective in control of mastitis. Administration of 3000 mg of Vit E 10 and 5 days prior to anticipated day of calving has been reported to decrease the incidence of mastitis. Similarly, administration of Vit E in the diet at the rate of 4000 IU during last 14 days and 2000 IU during lactation decrease the incidence of infection.

Application of Nanoparticles in Diagnostics

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The increasing presence of contaminants in food, air or drinking water that are capable of causing intoxication, diseases or chronic illness, has stressed the need for analytical systems capable of rapid multi-analyte measurements of complex samples. Similarly, multi-parameter diagnostic systems are increasingly required in order to detect all the well-known and the more recently appeared biomarkers for different diseases. When the detection system requires a bio-molecular recognition event, antibody-based detection methodologies are still considered the standard assays in environmental, food and clinical analysis. These assays are well established and they have been demonstrated to reach the desired sensitivity and selectivity. However, the use of antibodies in multi-analyte detection methods and in the analysis of very complex samples could encounter some limitations mainly deriving from the nature and synthesis of these protein receptors. In order to circumvent some of these drawbacks, other recognition molecules are being explored as alternatives.

Pathogen Recognition Elements

The recognition elements used in whole cell biosensors are generally biomolecules that have an affinity for epitopes present on the pathogen surface. A variety of recognition elements have been employed and include proteinaceous antibodies, nucleic acid aptamers, carbohydrates and antimicrobial peptides. In future years, inorganic recognition elements such as molecularly imprinted polymers (MIPs) may also have utility for pathogen recognition.

Antibodies. Antibody-based methods have been used extensively to detect bacteria, virus, toxins, and spores, alike. Highly selective and sensitive antibodies are readily available for many pathogens, and there are a number of well-established methods to conjugate antibodies to nanomaterials. For these reasons, immunological recognition by antibodies continues to be the most widely used tool for the selective capture and labeling of microorganisms. Three categories of antibodies are used in immunoassays: polyclonal, monoclonal and engineered antibody fragments. Polyclonal antibodies (pAbs) are produced *in vivo* and consist of a suite of antibodies that bind to a number of epitopes on the antigen. Monoclonal antibody (mAbs) solutions are produced *in vitro* from hybridoma cell lines and consist of an identical, well-defined population of antibodies that bind to a single epitope.

Carbohydrates. Carbohydrates (oligosaccharides or polysaccharides) are a large and diverse class of biomolecules that play an important role in dictating pathogen (and toxin) recognition and attachment to human cells. Many pathogens as well as their toxins recognize and bind to specific carbohydrate sequences in the glycocalyx. For example, type I fimbriae on *Enterobacteriaceae* bind to mannose terminated glycoproteins, influenza virus binds to *N*-acetyl neuraminic acid, and rotavirus binds to galactose.

Antimicrobial Peptides. Antimicrobial peptides (AMPs) play an integral role in the immune system response to pathogen infection. Consisting of sequences of 15-45 amino acids. AMPs recognize and semi selectively bind to microbial surfaces and, for reasons that are not completely understood, facilitate pathogen lysis. Similar to carbohydrates, AMPs exhibit a range of activity toward bacteria, virus, and fungi and have been suggested for use in sensor arrays that incorporate multiple AMPs. AMPs targeting Gram-negative bacteria bind nonspecifically to the negatively charged lipopolysaccharide (LPS) of both pathogenic and nonpathogenic organisms, while AMPs targeting Gram-positive bacteria often target peptidoglycan precursors required for synthesis of the bacterial cell wall.

Aptamers. Detection of biological terrorist threat agents, such as bacterial cells, spores, viruses and toxins, is a significant military and civilian challenge. Traditional analytical techniques for these targets are mainly based on immunological methods (Peruski and Peruski, 2003) such as conventional ELISA, immunomagnetic-electrochemiluminescence assays (Gatto-Menking *et al.,* 1995) or time-resolved fluorescence assays (Peruski *et al.,* 2002). However, in this particular field, aptamers can be of great advantage since all these methods are very dependent on the possibility of producing specific antisera for these toxic materials in animals. Aptamers due to their synthetic nature are independent of animals and they can be selected also for these toxic molecules. Aptamers specific for these particular targets, such as anthrax spores, cholera toxin, staphylococcal enterotoxin B, ricin and abrin toxin, have been selected in the last years (Bruno and Kiel, 1999, 2002; Kirby *et al*., 2004; Tang *et al*., 2007) and, by using these aptamers different detection systems have been developed.

Aptamers are single stranded DNA or RNA ligands which can be selected for different targets starting from a huge library of molecules containing randomly created sequences (Tombelli *et al.,* 2005) or peptide molecules that bind to a specific target molecule. Aptamers are usually created by selecting them from a large random sequence pool, but natural aptamers also exist in riboswitches. Aptamers can be used for both basic research and clinical purposes as macromolecular drugs. The selection process of Aptamers is called systematic evolution of ligands by exponential enrichment (SELEX), first reported in 1990 (Ellington and Szostak, 1990; Tuerk and Gold, 1990). The SELEX process involves iterative cycles of selection and amplification starting from a large library of oligonucleotides with different sequences (generally 10^{15} different structures). After the incubation with the specific target and the separation of the binding from the non-binding molecules, the oligonucleotides that are selected are amplified to create a new mixture enriched in those nucleic acid molecules having a higher affinity for the target. After several cycles of the selection process, the pool is enriched in the high affinity sequences at the expense of the low affinity binders.

The number of cycles required depends on the stringency conditions, but, once obtained and once the sequence is known, unlimited amounts of the aptamer can be easily achieved by chemical synthesis (Ngundi *et al.,* 2006). In addition to this very important aspect of having an unlimited source of identical affinity recognition molecules available, aptamers can offer advantages over antibodies that make them very promising for analytical applications (O'Sullivan, 2002; Luzi et al., 2003; You *et al*., 2003).

Recognition properties of Aptamers:

Conjugation of aptamers to either lipids or polymers such as polyethylene glycol improves their stability and distribution kinetics sufficient to produce therapeutic effects. The molecular recognition properties of aptamers are very similar to antibodies, which recognize a target with high affinity and specificity and in many cases effectively inhibit its function. Some of the best aptamers form complexes that have dissociation constants in the picomolar range, while many have dissociation constants that are similar to the antigen-binding fragment of antibodies. In terms of selectivity, aptamers can discriminate between very subtle structural differences, such as the presence or absence of a hydroxyl group or structural enantiomers (mirror images that have an identical chemical composition) of the target. Due to their relatively small size compared with antibodies, aptamers can fit into clefts where bulky molecules such as antibodies would otherwise be excluded. Their flexibility allows them to fold and assume the shape of relatively small binding pockets, thereby maximising surface contact with the target protein. These desirable properties of aptamers, combining the optimal characteristics of small molecules and antibodies, show great promise and have opened avenues for the development of therapeutic, antiviral, diagnostic and targeted drug delivery tools in areas that have been hitherto refractory to other approaches.

Advantages of Aptamers

1. The main advantage is the overcoming of the use of animals or cell lines for the production of the molecules.
2. Antibodies against molecules that are not immunogenic are difficult to generate, toxins and molecules that do not elicit a good immune response and are not suitable targets for immunotherapy can be used

as targets for the generation of high-affinity aptamers. Furthermore, aptamers are isolated by in vitro methods that are independent of animals: an in vitro combinatorial library can be generated against any target.

3. In addition, generation of antibodies *in vivo* means; the animal immune system selects the sites on the target protein to which the antibodies bind. The *in vivo* parameters restrict the identification of antibodies that can recognize targets only under physiological conditions limiting the extension to which the antibodies can be functionalized and applied.
4. Moreover, the aptamer selection process can be manipulated to obtain aptamers that bind a specific region of the target and with specific binding properties in different binding conditions.
5. After selection, aptamers are produced by chemical synthesis and purified to a very high degree by eliminating the batch-to-batch variation found when using antibodies. By chemical synthesis, modifications in the aptamer can be introduced enhancing the stability, affinity and specificity of the molecules. Often the kinetic parameters of aptamer–target complex can be changed for higher affinity or specificity.
6. Another advantage over antibodies can be seen in the higher temperature stability of aptamers; in fact antibodies are large proteins sensitive to the temperature and they can undergo irreversible denaturation. On the contrary, aptamers are very stable and they can recover their native active conformation after denaturation.

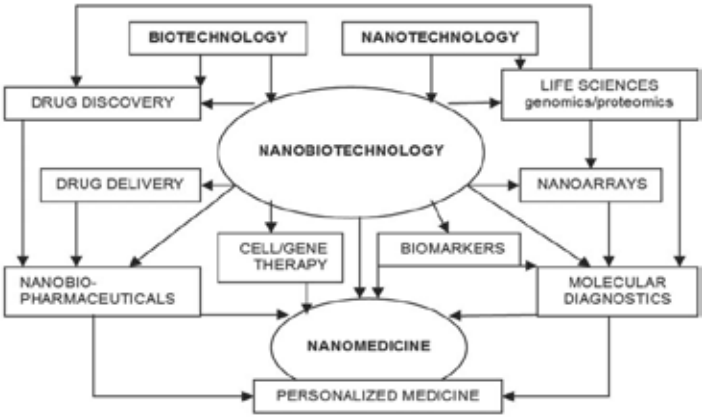
Limtations of Aptamers

The primary limitation on the use of aptamers (mainly RNA aptamers) in bioanalytical methods has been their nuclease sensitivity which is very critical for their use in ex vivo and in vivo applications (Famulok *et al*., 2000). However, it has been shown that the stability of such molecules can be improved by chemical modification of the ribose ring at the 20-position (Pieken *et al*., 1991). A different approach to stabilise aptamers comes from selection of RNA aptamers binding to stereoisomers of an intended target molecule, followed by chemical synthesis of the mirror-image of the selected sequences. As a consequence of molecular symmetry, the mirror-image aptamer (L-ribose) binds to the natural target molecule. Because of the substitution of the natural D-ribose with L-ribose, the mirrorimage aptamer is totally stable (Klussmann *et al*., 1996).

Nanoscience and Nano particles

Nanotechnology or nanoscience is a highly multidisciplinary field of applied science and technology intended to create, understand and use atomic and molecular scale (0.1-100nm) structures, and to fabricate devices or materials that lie with in the nano size range.

It applies the principles of engineering, electronics, physical and material science, and manufacturing to the molecular or submicron level.



This scheme shows the interrelationship of various technologies that contribute to clinical nano diagnostics. These technologies also contribute to development of nanomedicine under the concept of personalized medicine.

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm. The application of Nanotechnology to disease treatment, diagnosis, monitoring and to the control of biological systems has recently been referred to as nanomedicine. The medical uses of nanomaterials include advanced drug delivery systems, new therapies and *in vivo* imaging. It offers a suitable means of delivering small molecular weight NP drugs, as well as macromolecules, such as proteins, peptides or genes by either localized or targeted delivery to the tissue of interest.

Nano materials used for drug delivery must meet several requirements such as- biocompatibility, drug compatibility, suitable biodegradation kinetics, mechanical properties and ease of processing.

Multifunctional nanoparticle:

The nanoparticle's "corona" can be functionalized with hydrophilic polymers, targeting molecules, therapeutic drugs, and image contrast agents. The interior core can be solid (*e.g.,* quantum dots) or liquid (*e.g.,* liposomes).

Nanomaterials: Three different groups of nanotechnology materials are raw materials, nanostructured materials and materials such as nanotubes and fullerenes. The raw materials include nanoparticles and nanocrystalline materials which are more effective than bulk materials. Nanostructured materials are quantum dots and dendrimers. Nanotubes and fullerenes are 100 times stronger than steel, more conducive than copper and have several medical applications.

Commonly used nanomaterials are fullerenes, nanotubes, buckeyballs, quantum dots, dendrimers, nanoshells *etc.,* Nanomaterials compared to the macro materials can have very different properties. They can be stronger, lighter, more electrically conductive, more porous and less corrosive than bulk materials. They can even change colour *viz.,* gold can appear red, blue or gold depending on their size. Inorganic nonmaterial can detect electrical changes in biological molecules and help in detecting or treating a disease.

Buckeyballs: They are pure carbon molecules composed of 60-80 atoms of carbon. Because a fullerene takes a shape similar to a soccer ball or a geodesic dome, it is sometimes referred as Fullerene after the inventor of geodesic dome, Buckminister Fuller. In the buckeyball each carbon atom is bonded to three of its neighbours. They are mainly used in delivery of medicine or radioactive material to a disease site.

Nanotubes: Nanotubes are essentially buckey balls that have been opened on two sides with additional atom groups added in the characteristic hexagon shape to form a hollow carbon tube (cylinder). They are related to other form of carbons such as graphite and diamonds. They are sheets of graphite rolled into a cylinder. Nanotubes are also called buckeytubes.

Quantum dots: Among various nanomaterials, quantum dots (QDs) distinguish themselves in their far-reaching possibilities in many avenues of biomedicine. QDs are nanscale fluorescent semiconductor crystals with unique photochemical and photo physical properties. Their much greater brightness, rock-solid photo stability and unique capabilities for multiplexing, combined with their intrinsic symmetric and narrow emission bands, have made them far better substitutes for organic dyes in existing diagnostic assays.

Quantum dots are inorganic nanostructures composed of the same semiconducting materials used for developing computer chips two decades ago. In biological applications QDs have advantages over the traditional organic fluorophores due to their narrow, symmetric emission spectra, while requiring only a single excitation wavelength to simultaneously resolve multiple photostable colours. QDs have got three component layers where core is composed of semiconducting material such as cadmium selenide (CdSe) or cadmium sulphide (CdS) surrounded by a shell composed of zinc sulphide to protect the reactive core from pollutants which inturn is coated with a layer of orgnnic ligands to provide a hydrophilic outer surface for solubility in most biological buffers and a substrate for cross linking via reactive functional groups such as antibodies and oligonucleotides. The unique properties of QDs have enabled multiplexed

imaging of cellular targets for studying cancer biology, multiphoton fluorescence studies for deep tissue imaging in live animals and near-infrared imaging for sentinel lymph node (SLN) mapping at 1 cm tissue depth.

Dendrimers (Polymers): These are synthetic three dimensional man made macromolecules formed using a nanoscale fabrication. The unique features of the dendritic architecture include a high degree of structural symmetry, intramolecular minimum value and a well defined number of terminal groups.

Nanoshells: Nanoshells are colloids that consist of a core of non-conducting material covered by a thin metallic shell. By varying the thickness of metal shell, researchers can precisely tune the color of light to which the nanoshells respond. The infra red is suitable for whole blood immunoassay as it easily penetrates the whole blood well. When the antibody nanoshell particles are placed into a solution of whole blood containing the test molecules, it causes slight changes in the optimal properties of the nanoshells. By monitoring the changes, it is possible to detect the slight concentration of antigens in the blood.

Cantilevers: Cantilevers are made of silicon nitrite coated with gold on one surface, are mechanical beams anchored at one end and free standing at the other, similar to a swimming pool diving board. The cantilever bends in response to the change in surface stress upon binding of target molecule from a body fluid such as serum. The bending can be measured both optically and electrically which can be scaled up to an array format with as many as hundreds of cells for simultaneous detection of multiple biomarkers requiring minimal clinical samples.

Applications:

1. **Disease diagnosis:** Nanotechnology can be used in making cheaper, faster and more precise diagnostic tools. The nanotechnology can improve the quality of images produced by ultrasound machine. Nanoparticles injected into the breast can help the doctors to detect the cancers at very early stage. Nanotechnology based on the gold nanoparticles and DNA can detect prostrate specific antigen (PSA) in the blood when present at extremely low levels. This method could be used to detect prostrate cancer and to be used to monitor the prostrate cancer patients following surgery.

In the conventional immunoassay, whole blood cannot be used as it is so viscous and murky that it interferes with the chemical reaction in the test. By adopting nanotechnology, researchers have made it possible to test whole blood by using optically active gold-coated glass particles commonly known as gold nanoshells. The nanoshell immunoassay can detect less than one billionth of a gram of IgG in 1 ml of whole blood in 30 minutes.

Future blood tests may use tiny bar codes to speed up disease diagnosis like scanning a bar code of a grocery item. Unique DNA tags called bio-bar-codes can be used to detect the disease markers. The tags can be scanned by an instrument to identify disease starting from Alzheimer's disease to bio-terror agents such as anthrax, Ebola, Marburg or small pox. The test is easier, faster, more accurate and less expensive than PCR. The new test called bio-bar code amplification (BCA) could be ready shortly and a drop of blood is enough to screen the patient against a number of diseases.

1. **Treatment:** The nanoshells with targeted agent are injected into all animal and after a week animal's body is illuminated with infrared to raise the cell temperature to about 55^{o}C to activate the cancer killing agents to destroy the tumour. The smart superparamagnetic nanoparticles made up of iron oxides injected into the blood stream target tumour receptor cells when subjected to a magenetic field by emitting an attached drug. Quantum dots may also be injected into the blood stream of animals and upon stimulation with light capable enough to kill the cancerous cell. Nucleic acid engineered probes and methods offer powerful new ways to deliver therapeutics on preventive treatment for particular disease. The major challenge is to develop a non-viral DNA delivery system that has low toxicity and cost but high level of efficiency and specificity.
2. **Identity preservation:** Identity preservation (IP) system is a system that provides consumers with information about the practices and activities used to produce an agricultural product. Quality

assurance of the safety and security of agricultural products could be significantly improved through IP at the nanoscale level. Nanoscale IP has the potential to continuously track and record the history of a particular agricultural product. The keys are biodegradable sensors for temperature and other stored data to track all stages in the life of the product including the birth of the animal, its medical history, the slaughter house, meat packing plant, right through to the consumer's table.

3. Animal breeding: The management of breeding is an expensive and time consuming problem in dairy and swine industry. The nanotube implanted under the skin will provide the information about the level of estradiol in the blood during oestrous in animals by near infrared fluorescence. The signal from this sensor will be incorporated in a central monitoring and control system to inseminate the animals for improving the conception rate/breeding performance.

4. Drug delivery: Nanomaterials such as buckeyballs and dendrimers can be used in drug delivery systems. Buckey balls are inert, non-toxic perfectly smooth and can interact easily with cells, protein and viruses. Additionally they are hollow inside where drugs can be put so that it can release the drugs inside the cells. Dendrimers are synthetic polymers in various predetermined sized and can be used as delivery vehicle as it can hold a drug inside. They can enter cells .very easily and release drugs right on target. They do not trigger immune response and execute a five step task while dealing with the treatment of tumours (1) dendrimers may able to find tumour cells in the body by looking for tumour receptors (2) bind and pass through cell membrane (3) perform chemical analysis to know the type of tumour (4) release chemotherapy or radioactive agents inside the cells (5) confirmation of the death of tumor cells by chemical analysis.

Besides, targeting tumor cells the drug delivery systems, dendrimers showed promising results as tools in MRI and gene transfer techniques. Dendrimer based nanocomposites are being studied as possible anti-microbial agents against *Staphylococcus aureus, Pseudomonas aeroginosa* and *E. coli.* Nanotechnology has also entered the field of vaccinology. Synthetic oligonucleotides and antigens in bio-degradable nanospheres can be used as an alternative approach for immunization. A better immune response seems to be obtained with biodegradable nanospheres than with vaccines produced by conventional methods.

Now-a-days antibiotics, probiotics and pharmaceuticals are delivered to animals primarily through feed or injection. The medicine is delivered as a preventive measure or as a treatment once the disease organism has multiplied and symptoms are evident. Nanoscale devices have the capacity to detect and treat an infection, nutrient deficiency or other health problem long before symptoms are evident at the macroscale. This type of treatment could be targeted to the affected area and have multifunctional characteristics *viz.,* time controlled, spatially targeted, self-regulated, remotely regulated and pre--programmed. Smart delivery systems can also have the capacity to monitor the effects of the delivery of pharmaceuticals, neutraceuticals, nutrients, food supplements, bio-active compounds, probiotics, chemicals and vaccines.

Toxicity: The application of nanotechnology in different fields is not free from drawbacks. The particle size and surface are important characteristics when considering the toxicity of a material. As the size of the particle decreases, the surface area increases exponentially which allows for more potentially reactive groups to interact with the environment on the surface. It has been well established fine particles in the air can increase morbidity and mortality from pulmonary and cardiovascular diseases with long and short term effects. For example, exposure of human keratinocytes to carbon nanotubes was associated with oxidative stress and apoptosis. However, not all nanotubes are composed of same functional groups and nanomaterials with appropriate coating will have minimal toxicity. On the other hand, when injecting nanomaterials into humans as contrast agents, therapeutic carriers or sensors, one has to consider the rate of clearance. Iron oxide nanoparticles, for example, have been used as contrast agents and can be ingested by living cells and the biodegradation of the particles results in free iron that can be incorporated into Hb and the body is free of residues of iron oxide nanoparticles after months. Quantum dots encapsulated with the best protective shells will slowly break down in the body and eventually expose the core and release toxic ions.

Research needs in bovine mastitis

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Bovine mastitis, the most significant disease of dairy herds, has huge effects on farm economics due to reduction in milk production and treatment costs. Vehement research on bovine mastitis is comporting since past 7 decades but unfortunately the problem is still challengeable for the bovine mastitis researchers. Though considerable efforts have been expended in delineating its causes and control over the past years, a more in depth knowledge on distribution and changing trend of etiological agents within/between the different geographical region, pathobiology of the etiological agents, the interactions between bacteria and host, including the interaction between genotype and environment are essential to apply strategic plan for control of bovine mastitis.

Staphylococcal mastitis: A growing problem

Though research has been concentrated on the epidemiology, diagnostics, pathogenesis and treatment of mastitis caused by staphylococci and Coliforms, mastitis caused by staphylococci (particularly coagulase-negative staphylococci, or CNS) is a growing problem. To date, more than 50 Staphylococcus species and subspecies have been characterized. Although Coagulase-negative *Staphylococcus* species (CNS) are referred to as minor mastitis pathogens they are isolated frequently from bovine milk, the importance of CNS intramammary infections (IMI) has not been clearly delineated. The knowledge on CNS species involved in mastitis is still very limited and identification of CNS species has been unreliable.

With advances in methods of molecular biology benefits would accrue from having more reliable diagnostic methods for species identification. Prevalence of CNS varies from herd-to-herd and country-to-country as does the incidence of CNS clinical mastitis. Hence it is important to determine the predisposing factors for CNS mastitis at herd and cow levels. Epidemiological information on CNS is also needed to identify the best preventative strategies. Since CNS species are highly varied, there is a research need for their identification and understanding their pathogenesis, ecology and epidemiology in the herd and among individual cows.

To understand why some species/strains are more infectious, contagious and/or more resistant to treatment than other species/strains, knowledge on specific virulence factors is needed. Also in this area genotyping is an important tool. Some progress has been achieved in the understanding of important virulence factors for some pathogens. Further, the link between certain virulence factors and the severity and persistence of mastitis needs to be investigated as there is a relationship between antibiotic susceptibility and other virulence factors of the strain and the clinical features and prognosis of the disease. Research on virulence factors will aid in predicting the infection rate of different types of bacteria and in assessing treatment needs. Residual antibiotics in milk, staphylococcal enterotoxins and antibiotic resistance factors may also pose a risk to consumers, which is an important factor guiding the research.

Strengthening insights into host immune responses

To further understand the pathogenesis of intra-mammary infections, the interactions between bacteria and host, including the interaction between genotype and environment, requires further study. High-throughput genomic technologies, such as high density microarrays and sequencing-based tools [serial analysis of gene expression (SAGE) and massively parallel mRNA sequencing (mRNA-seq)], provide whole genome approaches to address such biological questions. In the last decade, gene expression profiling microarrays have been widely used in animal genomics and this technique has enabled researchers to monitor, on a broad scale, the effects of pathogens on host cells and tissues, aiming to gain insight into the molecular mechanisms that are involved in the host-pathogen interactions.

Numerous gene expression studies on mastitis in different host species infected with various pathogens are publicly available. However, due to the high costs of this approach, most individual studies have been carried out on limited numbers of technical and biological replicates. Thus, gene expression profiling studies of mastitis in ruminants have provided key but fragmented knowledge for the understanding of the disease. The majority of current studies on transcriptomic characterization focus on experimental infection, which may not accurately reflect the transcriptomic dynamics during a natural infection. Therefore, future studies should attempt to compare differential transcriptional response of various breeds and genotypes (either susceptible or resistant to mastitis) to natural IMI. Moreover, different pathogens induce specific transcriptomic responses apart from a general innate immune response. Thus, understanding the uniqueness of pathogen-specific responses is essential which would undoubtedly facilitate vaccine design and the development of effective intervention strategies.

Early detection of mastitis- A need of the hour

Early detection of mastitis and the identification of associated causative agents, will improve the well being of animals by allowing timely and efficient treatment. Various methods based on physical and chemical changes of milk are used for diagnosis of subclinical mastitis. Currently used diagnostic assays include measurement of SCCs, enzymatic analysis and the California milk clotting test. Mastitis can be also detected via changes in conductivity or pH though these effects are easy to monitor, they are relatively insensitive. Thus, there is a major need for new biomarkers that are specific for mastitis, easy to detect, occur at a very early stage and that can be measured 'on-site'. The development of novel analytical platforms incorporating enzymatic assays, immunoassays, biosensors are progressively replacing the more conventional methods.

The development of proteome profiles of mastitis causing pathogens, combined with available information on enzymes, toxins and metabolites produced in the udder could assist in identification of such biomarkers in the milk. A recent study compared mastitic milk sample with non-mastitic milk sample using direct liquid chromatography, tandem MS and 2D-GE followed by MALDI-TOF MS analysis and identified six chaperonins with a role of pathogen recognition exclusively in mastitic milk samples. These proteins thus have the potential to be developed as new markers for mastitis. This study also reported the presence of some neutrophils-associated proteins, cathelicidin, peptidoglycan recognition protein, lymphocyte cytosolic protein 1 and the macrophage scavenger receptor types I and II for the first time in milk samples. The application of biomarker based assays, developed within the last decade has already shown a considerable promise for mastitis detection. Yet additional studies on the validation of these assays for mastitis detection are required.

Also, immunoassays such as ELISA can be developed available against specific inflammation related biomarkers (especially present in different stages of subclinical mastitis) or causative microorganisms. Such immunoassays would provide a reliable and inexpensive approach for mastitis diagnosis. Biosensors have also been developed to detect mastitis. They use a biological receptor molecule like antibody, nucleic acid, enzyme etc. in combination with a transducer to produce an associated signal, allowing observation of a specific biological event like antigen antibody interaction. Further, recent advances in microfluidics will facilitate the development of improved technologies that could subsequently be incorporated into automatic monitoring systems and portable assays for sensitive and rapid detection of mastitis.

Bovine mastitis therapy

Research into treatment of bovine mastitis is quite traditional and seldom contains new approaches. Pharmacological and pharmacodynamic aspects should be considered more, as well as prudent use guidelines and risk of development of antimicrobial resistance. A weakness in many field studies on mastitis treatment is that results are not presented per bacterial groups, nor is in vitro susceptibility of the causative agents to the antibiotic reported. As milk is used for human consumption, antibiotic residue aspects should always be taken into consideration. Because of increasing societal pressure on use of antibiotics for disease prevention, the concept of selective dry cow therapy and use of non-antibiotic

strategies deserves more attention. Other points to consider include bacterium-related factors affecting treatment response, targeting treatment according to the causative agent, comparison of systemic versus intramammary therapy, application of supportive treatment and an optimal duration of treatment for different intra-mammary infections.

Vaccines against bovine mastitis

Approaches to enhance the cow's immunity to prevent disease and thus minimize use of antibiotics have gained considerable attention. Effective immunization against mastitis has been a goal of mastitis researchers for many years. Especially mastitis vaccines in developing countries are the urgent need to reduce the economic losses. Yet, for a variety of reasons, vaccines developed for the prevention and control of mastitis have achieved only limited success. The multiplicity of pathogens capable of causing mastitis, and insufficient knowledge of mammary gland immunology, bacterial virulence factors, and mechanisms of pathogenesis are factors that have hindered development of effective mastitis vaccines. Further, the large volume of milk (2–4 L/quarter/day during lactation) in the udder will invariably lead to dilution of the immune components available to fight infection and milk components such as fat and casein reduce the bactericidal abilities of the infection - fighting immune cells. Furthermore, some antibacterial factors which are active in serum lose their activity in milk.

Control of pathogens using immunological approaches requires identification of virulence factors and elucidation of their role in the pathogenesis of the infection. Equally important steps are validation of antigenicity in vivo and ubiquity of the virulence factor among strains of the pathogen. The availability of completely sequenced genomes of several pathogens allows elucidation of amino acid sequences and identification of dominant epitopes of newly discovered virulence factors. Use of this information, coupled with the availability of in vivo experimental approaches to test immune responses, vaccination timing, and protection against experimental infections will undoubtedly result in identification of virulence factors to be used in the design of vaccines for better control of mastitis and other diseases of food producing animals. Furthermore, identification and characterization of specific genes involved in mastitis resistance/susceptibility could result in new approaches of mastitis control through genetic selection. Additional research is needed to identify cow T- and B-lymphocyte response factors in relation to vaccination and experimental challenge that will provide direction for designing future vaccine formulations and therapeutic strategies.

Conclusions

Advances in mastitis research in the last decade have brought exciting new knowledge and technologies that can/will be used to solve complex problems confronting dairy production. New developments, approaches, strategies, and advances in mastitis diagnosis, treatment, and prevention can dramatically improve dairy herd health programs and result in reduced severity of mastitis, increased production and profitability of dairy farms, and ensure a supply of safe and nutritious dairy products for consumers throughout the world. It is for today's researchers to take up that challenge.

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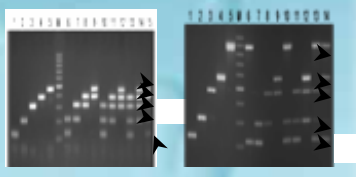
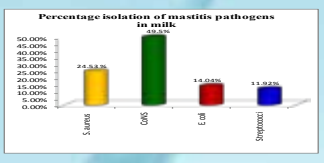
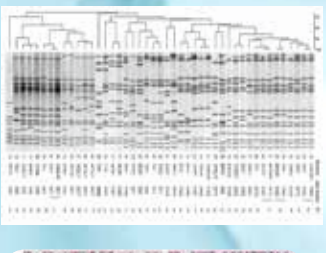
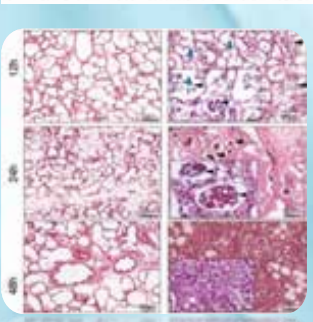




Mastitis Control... From Science to Practice!!





Take care of me…..
I shall take care of you
and your GENERATIONS…

Participants of MTC on "Bovine mastitis: Theoretical and Practical Consideration in Management" held from 1st to 8th March 2013