International Journal of Applied Research 2016; 2(6): 213-216

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 5.2
IJAR 2016; 2(6): 213-216
www.allresearchjournal.com
Received: 24-04-2016
Accepted: 25-05-2016

R Lakshmi
PhD Scholar, Department of Veterinary Biochemistry, College of Veterinary and Animal Sciences, Thrissur, Kerala, India.

Correspondence
R. Lakshmi
PhD Scholar, Department of Veterinary Biochemistry, College of Veterinary and Animal Sciences, Thrissur, Kerala, India.

Bovine mastitis and its diagnosis

R Lakshmi

Abstract
Mastitis is inflammation of mammary gland and most devastating disease condition in terms of economic losses occurring throughout the world. The etiological agents may vary from place to place depending on climate; animal species and animal husbandry and include wide variety of gram positive and gram negative bacteria; and fungi. Conventional diagnostic tests viz. California Mastitis Test (CMT) and Somatic cell count. The advent of Polymerase Chain Reaction (PCR) technology along with its various versions like multiplex and real time PCR has improved the rapidity and sensitivity of diagnosis. The measuring of the somatic cell count in milk is the standard method, but the analysis technique is problematic for routine use in herds. The most promising parameters for monitoring subclinical mastitis are milk N-acetyl--D-glucosaminidase activity, lactose, and electrical conductivity along with some other indicators such as optical and milk flow measurements, preferably with an inter-quarter evaluation included in the test. Acute phase proteins, haptoglobin and serum amyloid A, are also potential candidates for mastitis monitoring.

Keywords: Mastitis, diagnosis, CMT, SCC.

1. Introduction
Mastitis is defined as inflammation of the mammary tissue parenchyma, characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissue (Radostits *et.al,* 2000) [1]. Mastitis is one of the most prevalent diseases of high yielding dairy animals (Dhumka and Srivastava, 2003) [5]. Causing reduction in milk yield and qualit. Hence, it is of great economic importance to the dairy industry (Tollersrud *et al*., 2000) [6]. Mastitis is only second to foot and mouth disease as the most challenging disease in dairy animals (Sharma *et al*., 2007) [7].
There are two prevalent forms of mastitis in terms of level of severity; clinical, and sub-clinical. Clinical form of mastitis shows visible symptoms whereas sub-clinical form does not show any visible symptoms. Clinical mastitis in a dairy herd is threatening to a farmer but treatment can be given immediately to control it. While sub-clinical mastitis cannot be identified without a laboratory or field test, mostly remains unnoticed by the farmer.
The most frequently used diagnostic methods are California mastitis test, somatic cell counting (SCC) and bacteriological culturing (BC) of milk. Currently, methods such as measurement of N-acetyl-b-D-glucosaminidase (NAG-ase), lactate dehydrogenase (LDH), electrical conductivity (EC), and molecular methods such as polymerase chain reaction (PCR) technology are used less frequently. (Zadoks and Schukken, 2006) [8].

2. California Mastitis Test (CMT)
The California Mastitis Test is a simple, inexpensive, rapid screening test for mastitis. The test is based upon the amount of cellular nuclear protein present in the milk sample. Since inflammatory cells associated with mastitis is the predominant cell type present in milk. The CMT reflects the SCC level quite accurately and is a reliable indicator of the severity of infection. When milk and CMT reagent are mixed in equal amounts, the CMT reagent dissolves or disrupts the outer and nuclear cell wall of any leucocyte, which contain primarily fat. DNA, which is released from the nuclei will string or form gel together as a stringy mass. As the number of leucocytes increase in a quarter, the amount of gel formation will increase in a linear fashion (Table 1).

Table 1: CMT score to predict the somatic cell count of milk

CMT Score	Average somatic count (Cells per millilitre)	Description of reaction
N (negative)	100,000	No thickening, homogeneous
T (trace)	300,000	Slight thickening. Reaction disappears in 10 seconds
1	900,000	Distinct thickening, no gel formation
2	2,700,000	Thickens immediately, begins to gel, levels in the bottom of cup
3	8,100,000	Gel is formed, surface elevates, with a central peak above the mass

3. Somatic cell count

Measurement of number of somatic cells is the most common practice to identify cows with sub-clinical mastitis. Somatic cell count measure the number of leukocytes and other cells per millilitre of milk and from uninfected mammary gland should contain less than 200,000 cells/ml. In healthy udder, majority of somatic cell count (SCC) comprised of macrophages (66-88 per cent) followed by lymphocytes (10-27 per cent), neutrophils (1-11 per cent) and epithelial cells (0-7 per cent). An increase in SCC is also associated with decreased milk quality due to an influx of phagocytic cells, especially neutrophils. During mastitis, neutrophils (70 to 80 per cent) formed the major portion cell type in milk somatic cells and early influx of neutrophils resulted in early resolution of infection (Paape *et al*., 2002) [9]. Somatic cell count acted as the indirect indicator of udder health status in dairy cows.

4. Bacteriological culturing

Bacteriological culturing can be executed at herd, as well as cow and quarter level, each with its own specific goal. Bacteriological culturing is most often used as a diagnostic tool to solve mastitis problems. Knowledge on the infectious status of mammary glands, however, can also be very helpful to prevent transmission of pathogens by diagnosing a reservoir at an early stage. Additionally, historical BC results give herd-based information that can be helpful in optimising the treatment of future mastitis cases.

5. Molecular methods for diagnostic purposes

The use of molecular methods in pathogen detection has increased over the last years. Often, these methods use polymerase chain reaction (PCR) technology. Testing the presence of a specific bacterial species, a part of the DNA of that pathogen is amplified and subsequently visualised. For a number of mastitis pathogens, PCR-based techniques have been described (Lee *et al*., 1998; Baird *et al*., 1999) [11, 12]. The main advantage of PCR based assay is based on DNA and thus no matter of live or dead organisms which is crucial point for culture based detection but one disadvantages is that PCR detect lower number of organisms compare to culture methods. These methods are currently very labour-intensive and it is expensive to do a separate PCR test for every possible mastitis pathogen.

6. Multiplex PCR based diagnosis

Mastitis caused by multiple etiological agents and many times failure of the treatment is due to failure of real damage causing organism. Multiplex PCR also used for diagnosis of multiple pathogens in bovine mastitis milk samples (Phuektes *et al*., 2001) [14]. Multiplex PCR tests are of interest, in which several pathogens can be tested at the same time. The main drawback with multiplex PCR is that there is competition between different sets of primers for PCR substances like dNTPs and Taq polymerase which reduces the sensitivity (Amin *et al*., 2011) [15].

7. Real time based detection

Real-time PCR assays are being developed (Lightcycler, Taqman, Luminex, Biacore) for detection and quantifying mastitis pathogens in milk. Molecular methods can also be used to differentiate bacterial strains within one bacterial species. These differences may be of importance, because they may be associated with differences in virulence, epidemiology, and cure rates. For these purposes, phenotypic characteristics such as phage types, serotypes, and antibiotic sensitivity patterns can be used. Another possibility is to test for differences in strains by genotyping (fingerprinting) their genome.

8. Electrical conductivity

Mastitic milk has a higher electrical conductivity than normal milk. This is due to tissue damage and the subsequent increase in Sodium and Chloride ions in milk. Conductivity sensors are being incorporated in many new automated milking systems. The change in electrical conductivity is one of the earliest manifestations associated with new infections making the early detection and recording of possible mastitis cases routine. The greatest problem associated with this new technology is the sensitivity and specificity of electrical conductivity between herds. Recent advances in determining herd specific conductivity threshold levels have increased the value of this screening method in many herds. Milk conductivity is a screening test. A positive indication of increased electrical conductivity in a specific animal is an indication for further evaluation of that animal (temperature, udder examination, etc.) and not generally a signal for immediate treatment. Hand held conductivity meters are also available and may be useful for routine screening animals pre-milking.

9. Immune assay

ELISA based diagnostic method developed for *S. aureus* (Fox and Adams, 2000) [16], *Listeria monocytogenes* (Kalorey *et al*., 2007) [17], magnetic-bead-based ELISA for detecting Staphylococci using beads coated with an anti- *S. aureus* monoclonal antibody (Yazdan khah *et al*., 1998) [18]. Immunoassays also used for detecting inflammation-related biomarkers present in the milk at different stages of sub-clinical mastitis.

10. Proteomics based detection

Two-dimensional gel electrophoresis (2D-GE) and mass spectroscopy (MS) (Lippolis and Reinhardt, 2005; Smolenski *et al*., 2007) [19, 20] helped to identify various protein expressed during mastitis. These methods can be applied to detect the marker proteins from the cases of mastitis particularly from the acute, subacute and chronic mastitis.

11. Acute phase proteins for diagnosis of bovine mastitis

Acute phase proteins may provide an alternative means of monitoring animal health. Due to a relatively short halflife in serum and high response in diseased animals (Mackiewicz, 1997) [22], APP serum response constitute a valid measure of a systemic response to an initiating stimulus at the time of blood sampling. Like rectal temperature, APP levels are not suitable for establishing a specific diagnosis but can provide objective information about the extent of on-going lesions in individual's animals. At the herd level, APP might be useful for determining where the spread of the disease is taking place, by providing information about the prevalence of ongoing clinical and subclinical infections indicated by the high serum concentration of selected APP (Petersen *et al*., 2002) [21] and by serving as a prognostic tool, with the magnitude and duration of the acute phase response reflecting the severity of infection (Hirvonen *et al*., 1999) [23]. Haptoglobin, C-reative protein and serum amyloid A (SAA), which are the among the strongly reacting acute phase protein in animals.

C- reactive protein was discovered in the blood of patients during the acute phase of pneumococcal pneumonia (Tillet and Francis, 1930) [26]. In bacterial meningitis the CRP concentration was elevated, whereas no changes are seen in viral meningitis (Peltola, 1982) [25]. CRP is also reported to be useful for distinguishing between viral and bacterial pneumonia (McCarthy *et al*., 1978). Also, recent research has shown that slightly elevated CRP concentration might be a valid marker for increased risk of cardiac disease in humans (Ledue *et al*., 2003) [27]. Even though increased concentration of bovine CRP during naturally occurring infections and a correlation with herd health status have been reported (Lee *et al*., 2003) [29], CRP is generally not considered an acute phase protein in cattle (Nakajima *et al*., 1993) [28, 30].

In cattle, an increased SAA serum and plasma concentration has been found following experimentally induced (Bremner, 1964) [31]. and naturally occurring inflammation (Alsemgeest *et al*., 1995) [32] as well as experimental and natural infections. The SAA response during viral respiratory disease is well described (Ganheim *et al*., 2003) [33]. After inoculation with *Pasteurella multocida* the SAA concentration increased (Horadagoda *et al*., 1994) [34]. SAA has been suggested to be more useful in distinguishing between acute and chronic inflammation than neutrophil counts and white blood cells (Horadagoda *et al*., 1999) [35].

12. Cytokine prospects in the diagnosis of bovine Mastitis

In contrast to the poor outcome of cytokine immunotherapy for bovine mastitis, cytokines could provide a swift, reliable and highly sensitive means of diagnosis. Cytokines are the signals that dictate immune responses in normal and mastitis udders. Hence, subtle changes in the cytokine network of mammary gland in health and disease could help in detecting early infection and in monitoring the effectiveness of the treatment. The practical application of cytokines as a diagnostic means in bovine mastitis requires automation of the procedures for detecting and monitoring cytokines.

In terms of bovine mastitis, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor (Daley *et al*., 1993) [3], IFN-γ (Sordillo and Babiuk, 1991) [10], IL-1β (Daley *et al*., 1993) [3], IL-2 (Daley *et al*., 1993) [3] have been demonstrated to prevent, improve host clearance of, or enhance the efficacy of antimicrobial treatment of intramammary infections. A recent study, which demonstrated that intramammary infusion of LPS 24 h after *S. aureus* intramammary infection reduced milk bacterial concentrations during the period in which milk TNF-α concentrations were increased, further supports a role for cytokines in mediating the outcome of mastitis. In the case of *S. aureus* mastitis, for which 3 independent studies have established the lack of induction of major proinflammatory cytokines (Riollet *et al*., 2000) [2], administration of proinflammatory cytokines may be helpful in the treatment of these infections. However, in response to intramammary infections caused by pathogens such as *E. coli*, in which a highly proinflammatory state is evoked, and when exacerbated, can threaten the life of the cow, administration of anti-inflammatory cytokines may be beneficial (Sordillo and Peel, 1992) [4]. Because of the pathogen-dependent differences in the kinetics and magnitude of cytokine responses, cytokine therapy may need to be tailored to individual pathogens to be successful. In addition to their potential therapeutic application, cytokines may be useful biomarkers of disease severity and outcome.

13. Conclusion

In general mastitis is a condition which is at present among the most severe damage causing conditions to dairy industry. The economic losses due the conditions are beyond repair and this is due to late and improper diagnosis of main etiological agent. Much knowledge in the field of mastitis diagnostics is available and science is still developing. Several diagnostic methods are available to monitor the effect of interventions in udder health, but these are not always fully exploited. Although there are many advanced tests available for the diagnosis but the core issue is early and effective diagnosis as the losses occurs so quickly that the delay of few hours can be the loss of completer teat or udder. In such conditions mastitis can be handled best by two means, first one through continuous monitoring with routine examination of physical condition of udder, milk and examining the quality of milk. Secondly use of disinfectants and available vaccines in endemic areas on regular basis.

References

1. Radostits OM, Gay GC, Blood DC, Hinchcliff KW. Vet. Med. 9th edn, ELBS, Bailliere, Tindal. 2000, 563-618.
2. Riollet C, Rainard P, Poultrel B. Kinetics of cells and cytokines during immune-mediated inflammation in the mammary gland of cows systemically immunizedwith Staphylococcus aureus α-toxin, Inflamm. Res. 2000; 49:486-492.
3. Daley JM, Williams TJ, Coyle PA, Fruda G, Dougherty R, Hayes PW. Prevention and treatment of Staphylococcus aureus infections with recombinant cytokines. Cytokine. 1993; 5:276-284.
4. Sordillo LM, Peel JE. Effect of interferon-gamma on the production of tumor necrosis factor during acute Escherichia coli mastitis, J Dairy Sci. 1992; 75:2119-2125.
5. Dhumka VK, Srivastava AK. Pharmacology of antibiotics used in the treatment of mastitis: An overview. Proceedings on 4th round table on mastits, IVRI, Izatnagar. 2003, 97-111.
6. Tollersrud T, Kenny K, Reitz AJ, Lee JC. Genetic and serologic evaluation ofcapsule production by bovine

mammary isolates of Staphylococcus aureus and other Staphylococcus spp from Europe and the United States, J Clin. Microbiol. 2000; 38:2998-3003.
7. Sharma N, Maiti SK, Sharma KK. Prevalence, Etiology and antibiogram ofmicro-organism associated with sub-clinical mastitis in buffaloes in durg, Chhattisgarh state (India). Int. J Dairy Sci. 2007; 1:7-12.
8. Zadoks RN, Schukken YH. Use of molecular epidemiology in veterinary practice, Veterinary Clinics of North America Food Animal Practic. 2006; 2:229-261.
9. Paape M, Mahrzad J, Zhaom X, Detilleuxm J, Burvenich C. Defence of the bovine mammary gland by polymorphonuclear neutrophil leukocytes. J Mammary Gland Biol. Neoplas. 2002; 7:109-121.
10. Sordillo LM, Babiuk LA. Controlling acute Escherichia coli mastitis during the periparturient period with recombinant bovine interferon gamma, Vet. Microbiol., 1991; 28:189-198.
11. Lee SU, Quesnell M, Fox LK. Characterisation of staphylococcal bovine mastitis isolates using the polymerase chain reaction, J Food Protec. 1998; 61:1384-1386.
12. Baird SC, Carman J, Dinsmore RP. Detection and identification of Mycoplasma from bovine mastitis infections using a nested polymerase chain reaction, J Vet. Diagn. Invest. 1999; 11:432-435.
13. Yoshimura A, Lien E, Ingalls RR, Tuomanen E, Dziarski R, Golenbock D. Recognition of Gram–positive bacterial cell wall components by the innate immune system occurs via Toll–like receptor 2, J Immunol., 1999; 163:1-5.
14. Phuektes P, Mansell PD, Browning GF. Multiplex polymerase chain reaction assay for simultaneous detection of Staphylococcus aureus and streptococcal causes of bovine mastitis. J Dairy Sci. 2001; 84:1140-1148.
15. Amin AS, Amouda RHH, AAA, Abdel-All. PCR assays for detecting major pathogens of mastitis in milk samples. World J Dairy Food Sci., 2011; 6:199-206.
16. Fox LK, Adams DS. The ability of the enzyme-linked immunosorbent assay to detect antibody against Staphylococcus aureus in milk following experimental intramammary infection. J Vet Med B. Infect. Dis. Vet. Public Health. 2000; 47:517-526.
17. Kalorey DR, Kurkurey NV, Warke SR, Barbuddhe SB. Evaluation of indirect and avidin-biotin enzyme linked immunosorbent assays for detection of anti-listeriolysin O antibodies in bovine milk samples. Zoonoses Public Health, 2007; 54:301-306.
18. Yazdan Khah SP, Hellemann AL, Ronningen K, Olsen E. Rapid and sensitive detection of Staphylococcus species in milk by ELISA based on monodisperse magnetic particles. Vet. Microbiol, 1998; 62:17-26.
19. Lippolis JD, Reinhardt TA. Proteomic survey of bovine neutrophils. Vet. Immunol. Immunopathol. 2005; 103:53-65.
20. Smolenski G, Haines S, Kwan FY, Bond J, Farr V. Characterization of host defence proteins in milk using a proteomic approach. J Proteome Res. 2007; 6:207-215.
21. Petersen HH, Dideriksen D, Christiansen BM, Nielsen JP. Haptoglobin serum concentration as marker of clinical signs in finishing pigs, Vet. Rec. 2002; 151:85-89.
22. Mackiewicz A. Acute phase proteins and transformed cells, Int. Rev. Cytol., 1997; 170:225-300.
23. Hirvonen J, Eklund K, Teppo AM, Huszenicza G, Kulcsar M, Saloniemi H *et al*. Acute phase response in dairy cows with experimently induced Escherichia coli mastitis, Acta Vet. Scand. 1999; 40:35-4.
24. Mccarthy PL, Frank AL, Ablow RC, Masters SJ, Dolan TF. Value of C-reactiveprotein test in the differentiation of bacterial and viral pneumonia, J Pediatr., 1978; 92:454-456.
25. Peltola HO. C-reactive protein for rapid monitoring of infections of the central nervous system, Lancet, 1982, 980-982.
26. Tillet WS, Francis T. Serological reactions in pneumonia with a non-protein somatic fraction of Pneumococcus, J Exp. Med. 1930; 52:561-571.
27. Ledue TB, Rifai N. Preanalytic and analytic sources of variations in C-reactiveprotein measurement: Implications for cardiovascular disease risk assessment, Clin. Chem., 2003; 49:1258-1271.
28. Nakajima Y, Momotani E, Murakami T, Ishikawa Y, Morimatsu M, Saito M, *et al*. Induction of acute phase protein by recombinant humaninterleukin-6 in calves, Vet. Immunol. Immunopathol. 1993; 35:385-391.
29. Lee JW, Paape MJ, Elsasser TH, Zhao X. Recombinant soluble CD14reduces severity of intramammary infection by Escherichia coli, Infect. Immun. 2003; 71:4034-4039.
30. Nakajima Y, Momotani E, Murakami T, Ishikawa Y, Morimatsu M, saito M, *et al*. Induction of acute phase protein by recombinant humaninterleukin-6 in calves, Vet. Immunol. Immunopathol. 1993; 35:385-391.
31. Bremner KC. Studies on haptoglobin and haemopexin in the plasma of cattle, Aust. J Exper Biolo Med Sci. 1964; 42:643-656.
32. Alsemgeest SPM, Lambooy IE, Wierenga HK, Dieleman SJ, Meerkerk B, Van Ederen AM *et al*. Influence of physical stress on the plasma concentration of serum amyloid A (SAA) and haptoglobin in calves, Vet. Q. 1995; 17:9-12.
33. Ganheim C, Hulten C, Carlsson U, kindahl H, Niskanen R, Waller KP. The acutephase response in calves experimently infected with bovine viral diarrhea virus andor manheimia haemolytica, J Vet Med Ser B. 2003; 50:183-190.
34. Horadagoda A, Eckersall PD, Hodgson JC, Gibbs HA, Moon GM. Immediateresponse in serum TNF alpha and acute phase protein concentration to infectionwith Pasteurella haemolytica A1 in calves, Res. Vet. Sci. 1994; 57:129-132.
35. Horadagoda NU, Knox KMG, Gibbs HA, Reid SWJ, Horagoda A, Edwards SER *et al*. Acute phase proteins in cattle: discrimination between acuteand chronic in flammation, Vet. Rec. 1999; 144:437-441.