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Updates in Bovine Mastitis

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Abstract: Mastitis is one of the most common and economically important diseases in dairy cows in the world. The disease is considered as an important welfare issue facing the dairy industry. The objective of this review article was to summarize the most recent clinical field trials that have been published including the prevalence, economic value, risk factors, antimicrobial resistance, recent methods for diagnosis, evaluating the use of different types of vaccines against mastitis pathogens, recent studies to Explore the genetic architecture and improve genomic prediction accuracy for mastitis and milk production traits in dairy cattle.

Key words: Mastitis · Immunization · Resistant pathogens · Genetic · Diagnosis · Control

INTRODUCTION

Clinical mastitis is a common and economically important disease in dairy animals because of reduced milk production, altered milk quality, increased involuntary culling rates and post treatment disposal of milk [1]. Also, there is evidence indicate that the effect of mastitis is not restricted to udder, but also extend to reproductive organs, thereby affecting the reproductive efficiency in dairy cattle. It lowers the pregnancy rates and cause aberrations in the estrous cycle, early embryonic mortality or abortions, prolonged days open, higher number of services per conception and decreased conception rate in dairy cattle [2].

This work is done to update the published work about mastitis concerning the incidence in different countries, risk factors, treatment and antimicrobial resistance and modern methods of diagnosis and control

Incidence: The incidence of clinical mastitis is an important indicator of animal health and welfare. The decrease in incidence of clinical mastitis has a positive effect on animal health, animal welfare, antimicrobial use, work pleasure and net return of the farm [3]. The incidence of clinical mastitis were found to be 11.5% in India and recorded *Staphylococcus aureus* as the major causative agent (60.87%) followed by coagulase negative Staphylococci (13.04%), *Streptococcus uberis* (4.35%), *Streptococcus dysgalactiae* (8.69%) and *Escherichia coli* (13.04%) in bovines [4], 74.7 and 62.6% in Ethiopea at the herd-level and cow- level, respectively and the later showed 59.2 and 3.4% sub-clinical and clinical mastitis cases, respectively [5]. In Tanzania, the overall prevalence of subclinical mastitis was 28.6, 48.8 and 64.7% at quarter, cow and farm level, respectivelyand the prevalence of bacterial infection was recorded as 36.8, 17.8, 16.1, 9.5, 6.3 and 4.9 % for *Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, Klebsiellaspp, Micrococcus spp and Escherichia coli,* respectively [6].

Streptococcus agalactiae was reported as the predominant cause of bovine mastitis in Denmark with prevalence as 27.4% based on PCR and 7.8% based on bacteriological culture on cow-level [7].

In Egypt, the prevalence of subclinical mastitis, in 60 smallholder dairy farms (340 milking cows and buffaloes) in Ismailia, was 71.6 and 43.5 % in cattle and buffaloes, repectively at farm level (based on CMT) and 25.2 and 21.7 % at cow and buffaloes-quarter level, respectively. The most frequently identified bacteria were *Staphylococcus aureus* and *Streptococcus agalactiae* (38.3 and 20 %) based on bacterial culture and (41 and 22.6 %) based on PCR, respectively.Furthermore, methicillin-resistant *S. aureus* (MRSA) strains occurred in52.2 and 45 % of isolates of animals and workers, respectively [8]. Also, in Denmark, when 1199 cattle from 6 herds *were* examined, *Streptococcus agalactiae*was

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reported as the predominant cause of bovine mastitis at cow-level with estimated prevalence 27.4% based on PCR and 7.8% based on bacteriological culture [7].

Outbreaks of mycoplasma mastitis have been reported in some European countries like Denmark [9], Austria [10], The Netherlands [11], Switzerland [12] and, more recently, Norway, which, until 2014, had been *M. bovis*-free. The prevalence of mycoplasma mastitis was 1.5% in Belgium [13] and 5.4% in Greece [14] while this kind of mastitis was much higher in other countries (55% and 100% in Mexico and Iran, respectively) [15, 16]. Recent surveys in Australia indicated that the prevalence of *M. bovis* was low in dairy herds [17], while New Zealand was probably free of *M. bovis* [18]. In the USA, the prevalence of mycoplasma ranged from < 3% of bulk milk tanks in the Northeast and Midwest to 9.4% in the large dairy herds in the West [19].

Economic Importance: Costs due to mastitis include reduced milk production, condemnation of milk due to antibiotic residues, veterinary costs, culling of chronically infected cows and occasional deaths [20]. Moreover, mastitis has a serious zoonotic potential associated with shedding of bacteria and their toxins in the milk [21]. the negative effect of mastitis on the reproductive performance of the animals add to economic losses. The economic losses due to mastitis in Australia and New Zealand was estimated by more than $130 million ($200/cow/year) and NZ$300 million annually respectively [22].

In India, annual economic losses due to subclinical and clinical mastitis have been estimated to be INR. 41.511 and INR 30.144 billions, respectively, with a total of INR 71.655 billion (1 USD = 64.8 INR) [23].

The effect of mastitis on cows reproductive performance will add to its economic importance where the low fertile cow have low calf crop, low milk yield, more veterinary service and treatment cost [24]. This pathogenesis is affected by some factors like the time of mastitis occurrence (before or after AI), type and virulence of pathogens and severity or duration of infection (acute severe or chronic subclinical, etc.). Recently, it was observed that the occurrence of mastitis prior to and/or after first service resulted in extended days open in Zebu cattle and Murrah buffaloes [25].

How Mastitis Can Affect on Reproductive Efficiency ?: Mastitis leads to elevated inflammatory mediators, cytokines and nitric oxide concentration. These inflammatory mediators will affect directly on hypothalamic-pituitary axis function (altered GnRH, FSH and LH secretion) and increase the body temperature. This consequently affects on ovarian function (disruption in oocyte maturation, delayed ovulation, poor CL development, premature leutolysis and decreased progesterone production) which will result in uterine PGF2α release, early embryonic mortality, abortion, altered cyclicity and repeat breading [26]. Several authors reported that the gram positive or gram negative characteristics of clinical mastitis pathogens did not differ in their effects on reproduction [27, 28]. Also, both chronic subclinical and acute clinical mastitis resulted in delayed ovulation associated with low plasma estradiol and a low or delayed preovulatory LH surge [29].

Risk Factors: The incidence of clinical mastitis is associated with many risk factors and the sampling unit in risk factor studies can vary from quarter level to herd level.

Cow-specific risk factors means the difference in clinical mastitis incidence among cows. Parity, month of lactation, season of the year, somatic cell count in previous lactation and clinical mastitis history are the cow-specific risk factors that are currently known [30].

***Microbial Risk Factors*:** A wide variety of microorganisms including bacteria, fungi, yeast and mycoplasma are responsible for causing mastitis, of which bacteria are the most frequently isolated pathogens. The mastitis causing pathogens can be classified as contagious and environmental pathogens. The major contagious pathogens comprise *Streptococcus agalactiae, Staphylococcus aureus* and *Mycoplasma bovis* and the major environmental pathogens include *Enterobacteriaceae*(particularly *Escherichia coli*) and *Streptococcus uberis*. Coagulase negative *Staphylococci* infections tend to be subclinical and coliform infections tend to be clinical [31].

The causative organism differs according to locality. In India, Tanzania and Egypt, *Staphylococcus aureus* was recorded as the major causative agent of clinical mastitis [1, 4, 8]. While in Denmark, *Streptococcus agalactiae* was reported as the predominant cause of bovine mastitis [7].

Mycoplasma bovis is the main reason of mastitis in Mexico and Iran [15, 16] while its incidence is much less in Europe, Australia and New zealand.

Mastitis caused by mycoplasmas is less common than mastitis caused by other bacteria, but results in severe udder disease. It is often refractory to antibiotic treatment;

may result in an increase in purulent mastitis. Affected cows can remain externally normal, with few overt clinical signs, even in severe cases [19].

The risk factors associated with *M. bovis* mastitis was the herd size where the larger herds (> 500 cows) were more vulnerable to mycoplasma mastitis than smaller herds. Also, when infected animals is introduced into a herd, mycoplasma can be transmitted rapidly to up to 40% of healthy cattle unless they are segregated [32]. Cows with mastitis can shed > 1 x 106 mycoplasmas/mL via milk [33]. In the same time, Feeding waste milk or colostrum to livestock increases the risk of transmission to the rest of the herd and may cause otitis in calves [34]. The presence of calves in close contact with dairy cattle is considered as a risk factor [35].

***Genetic Risk Factors*:** Mastitis resistance and milk production are typical complex traits controlled by a minimum of 400–4000 effective loci in cattle [36].

Studying the genetic architecture (the distribution of causal variants and their effects on genomic polymorphysm) and predicting future individual phenotypes for complex traits and diseases are very important in evolution of animal breeding.

Recently, Fang *et al*. [37] studied the association between some gene ontology (GO) terms for different biological processes with the four traits of milk production (protein, milk and fat yields) and mastitis in a Holstein cattle population. Results of analysis using GWAS(global wide association analysis studies) showed that milk production was highly associated with five GO terms (positive regulation of multicellular organism growth, retinol metabolic process, response to lipopolysaccharides, positive regulation of transcription from RNA polymerase II promoter and cellular response to heat stress).

For mastitis, five predictive GO terms (negative regulation of apoptotic process, response to lipopolysaccharides, negative regulation of protein binding, positive regulation of cysteine-type endopeptidase activity involved in apoptotic process and cellular response to interferon-gamma) have been suggested to be associated with mastitis.

The response to Lipopolysaccharides (*E. coli* LPS) was highly predictive of both milk production and mastitis thus revealed that several immune relevant pathways (e.g., chemokine signalling pathway and leukocyte transendothelial migration) were significantly associated with milk production and mastitis and reflect the genetic correlation between both traits [38].

Because liver is a crucial organ for host immune responses and metabolism, including lipogenesis, gluconeogenesis and cholesterol metabolism, so that the immune responses in the liver during mastitis impair the physiological process for milk, fat and protein synthesis [39, 40].

Earlier studies reported some genetic variants underlying resistance or susceptibility to mastitis in dairy cows and heifers like genes of the major histocompatibility complex (MHC) or BoLA system. In cattle, the BoLA system is located on chromosome 23 and includes three classes: class I (locus A); class II, divided into IIa (includes loci DRA, DRB1-3, DQA, DQB, etc.) and IIb (includes DOB, DYA, DYB, DIB, etc.); and class III (includes TNF, 21-OH, C4, BF, HSP70-1 and -2, EAM, PRL, etc.) [41]. The first two groups of loci code for surface molecules relevant in the induction and regulation of immune responses. Some of these genes are highly polymorphic, with about100different alleles described for DRB3exon 2 and about 39 for DQB. In dairy cattle, exon 2 of the class II BoLA DRB3 was reported to have high polymorphism and associated with mastitis [42, 43].

Using PCR-RFLP method and PCR-sequence-based typing (PCR-SBT). The frequencies of DRB3.2*8 and DRB3.2*16 associated with susceptibility to mastitis. While, DRB3.2*22, DRB3.2*23 and DRB3.2*24 was higher in healthy cows than in mastitic cows [44].

Toll like receptor gene (TLR) showed significant association between the SNPs and somatic cell score (SCS) in dairy cattle. Animals with CC genotype were found to be significantly lower than that of the TTgenotype Investigation of SNPs in TLR4 gene is associated with subclinical mastitis in Holstein cows using TaqManallelic discrimination. Animals with combined genotypes AACCCC, GGTCGG and GACCGC had the lowest somatic cell scores. These genotypes were predicted to have the potential to be applied as molecular markers for assisted animal selection to improve milk quality [45].

Lactoferrin gene showed tight relationship with mastitis in Chinese Holstein cattle. Results of PCR-RFLP showed significant association between combined genotypes of three SNPs, haplotype and SCS [46].

Microarray and real time PCR analysis have become important tools in animal genomics as this high throughput technology can offer the possibility of studying changes in expression profiles of thousands of genes, in response to infection with a pathogen [47]. Eleven genes (IL6, IL8, CD14, TLR4, IL1B, LBP, TLR2, C5AR1, TNF, IFNG and SAA3) showed high expression

(two to four times) during mastitis. These findings suggest that animals with mastitis develop a preferentially cell-mediated immune response [48, 49].

Treatment and Antimicrobial Resistance: Recent study in Egypt showed the dominance of *S. aureus* in 42% of examined mastitic milk. The isolated strains (70) revealed a high resistance against ampicillin (95.2%) and penicillin (83.3%) and a lower resistance was observed against gentamicin (23.8%), amikacin (16.7%) and ciprofloxacin (14.3%). Multi drug resistances were detected in 83.3% of the isolated *S. aureus*. Out of the penicillin-resistant *S. aureus* isolates, β-lactamases (blaZ gene) was identified in 95.7% isolates. Fifty percent of *S. aureus* isolates harbored the specific amplicon of mecA gene. Markedly, all mecA positive strains displayed multidrug resistance and were also positive for blaZ gene. The virulence determinants pvl and tst were detected in 7.1 and 11.9% of the isolated *S. aureus*, respectively. It was concluded that the presence of multidrug resistant and toxin producing *S. aureus* in dairy farms pose a major risk to public health [49].

Recent study in indea recorded the best antimicrobial drugs (individual and combined) were enrofloxacin, gentamicin, amoxicillin/sulbactam, ceftriaxone/tazobactam, ceftizoxime, ampicillin/sulbactam against Staphylococcus aureus, coagulase negative Staphylococci, Streptococcus uberis, Streptococcus dysgalactiae and *Escherichia coli* which were isolated from clinical mastitis cases in bovines [50].

Another study was held in New Zealand and USA to compare the antimicrobial susceptibility patterns of three common mastitis pathogens (*Staphylococcus aureus, Streptococcus uberis and Str. dysgalactiae*) to study the value of national surveys to determine the susceptibility patterns of mastitis pathogens. All isolates were susceptible to the amoxicillin–clavulanic acid combination. Resistance to lincomycin was most frequent (susceptibility of 8.6%) across all species. Non-susceptible isolates of *S. aureus* were identified for lincomycin (99.9% and 94.6%) and the three non isoxazolyl penicillins (amoxicillin, ampicillin and penicillin) as 20.6% and 36.0% for NZ and the USA isolates, respectively. Resistance to erythromycin and tetracyclines was detected only in the USA isolates. There were differences in susceptibility between *Str. uberis*and *Str. dysgalactiae*; all streptococcal isolates demonstrated resistance to aminoglycosides (neomycin 52.4% and streptomycin 27.9%) and enrofloxacin (28%). Resistance of *Str. dysgalactiae*to tetracycline was almost 100.0% and to oxytetracycline 89.9%.

Differences of antimicrobial susceptibility of the isolated pathogens between NZ and the USA confirm the value of national surveys to determine the best treatment of mastitis [51].

Diagnosis: Effective prevention of bovine mastitis requires an understanding of the infection status of a pathogenic microorganism in a herd that has not yet shown clinical signs of mastitis and appropriate treatment specific for the pathogenic microorganism. However, bacterial identification by culture has drawbacks in that the sensitivity may be low and the procedure can be complex.

Recently, Kawai *et al*. [52] developed new method to identify mastitis pathogens using a simple and highly sensitive electrochemical DNA chip which can specifically detect bacterial DNA in milk specimens. First, microorganisms belonging to 12 families and/or genera associated with mastitis were selected. Amplifying their DNA by loop-mediated isothermal amplification (LAMP) using 32 primers and the use of a DNA chip capable of measuring all pathogens simultaneously.

Sample detection could be completed in just a few hours using this method. Comparing the results obtained with the DNA chip method and by bacterial culture verified that when the culture method was set to 100%, the total positive concordance rate of the DNA chip was 85.0% and the total negative concordance rate was 86.9%. Furthermore, the proposed method allows both rapid and highly sensitive detection of mastitis pathogens.

Control Measures: Strategies aimed to control intramammary infections include improved hygiene and teat health [53] as well as proper milking procedures, which are important to reduce exposure to pathogens causing mastitis [54]. Dry-cow antibiotic therapy is a protocol in many dairy herds to treat the present infections and is considered as a prophylactic to prevent new infections in the dry period and after calving [55]. Great success has been achieved in controlling mastitis caused by infectious pathogens, but the incidence of mastitis caused by environmental pathogens *(E. coli and Klebsiella, Enterobacter and Citrobacter spp.)* remains problematic. Antibiotic therapy is not always successful in resolving infections. Vaccine development against common udder pathogens has been advancing in the past few decades. The results of efficacy studies of these vaccines have been controversial or modest at best. The most commonly targeted udder pathogens were *S. aureus*, *S. agalactiae* and *E. coli*. Vaccines against *S. aureus*and *S. agalactiae*contained either the whole organism (cellular

lysates, inactive and attenuated vaccines) or subunits (toxins, surface proteins and polysaccharides) while for *E. coli*, the mutant core antigen J5 was used most commonly. Vaccines were also classified as mono or polyvalent according to the number of targeted pathogens it contained [56] and classified according to the field of use to marketed, autogenous (herd-specific) and experimental vaccines. The commercially available polyvalent vaccine (Startvac, Hipra, Spain) containing *E. coli J5* and *S. aureus*strain SP 140 [57].

Recent study in Germany showed that the prevalence of *S. aureus* mastitis was significantly lower in cows that were vaccinated using the commercial and herd-specific vaccines compared to the controls but the 305-days milk production was significantly less in vaccinated groups [57].

Other studies has been done under UK and Swedish field conditions, cows from different dairy farms were administered the polyvalent commercial vaccine (Startvac) and there was significant reduction in the severity of clinical mastitis and significantly more milk and milk solids than unvaccinated cows. The net return of investment between groups was approximately 2.5:1 due to increased milk yield alone[58]. Using the same vaccine in Italy and Spain Showed marked reduction in the duration of mastitis and the incidence of new mastitis cases due to S. aureus and coagulase-negative Staphylococci, lower SCC and polymorphonuclear cell counts, significantly more serum specific *S. aureus*and J5 IgG concentrations and less drop in milk production in vaccinated compared to non-vaccinated animals [59, 60].

Evaluation of the protective effect of a commercial vaccine (J5) containing E. coli rough mutant O111:B4 bacteria was done in dairy farms in Canada and USA. Vaccinated cows showed higher level of IgG1 and IgG2 than in control ones [61].Also, vaccination with the herd-specific autovaccine prepared from S. aureus JR3 cells and SM capsule of the strain S. aureus 2286 showed significant reduction in the rate of subclinical and clinical mastitis in the dairy cows [62].

In another study, the efficacy of a herd-specific autovaccine alone or in combination with intramammary antibiotic therapy (cefuroxime) in eliminating S. aureus infection in a herd with high subclinical mastitis rate was evaluated. The combination of the antibiotic/autovaccine was an effective method and clinical mastitis was not recorded in those vaccinated cows for at least 2 years [63]. However, vaccination as a tool used to prevent mastitis is not necessarily effective or economical especially in dairy herds with high mastitis rates. Regardless of the type of used vaccine, the combination of vaccination with antibiotics and the application of other infection control procedures, such as excellent milking hygiene procedures, isolation of clinical cases and culling of known infected cows are important preventative measures that usually result in a significant reduction in the incidence and duration of intramammary infections [56].

Improve Genomic Prediction for Mastitis and Milk Production: Selection of animals genetically resistant to mastitis for subsequent breeding is an area of research gaining momentum, with data suggesting that enhanced expression of certain antimicrobial genes increase resistance to mastitis. A better understanding of the genetic architecture of complex traits can contribute to improve genomic prediction by modifying genomic variants. Since liver plays key roles in innate immune response and metabolic regulation [64]. It was hypothesized that hepatic transcriptomic regions that are responsive to intra-mammary infection (IMI) may be enriched in genomic variants (single nucleotide polymorphisms (SNPs)) that may improve the predictive ability of a genomic model. Fang *et al*. [65] studied the sequence-level genotype variants of mastitis resistance and three milk production traits (milk, fat and protein yields) of different cattle breeds (Nordic Holstein, Nordic red cattle and Jersey) involving two tissues (liver and mammary gland) after itramammary infection (IMI) with two pathogenic factors (E. coli and E. coli endotoxin (LPS). They concluded that genes of mammary gland were preferentially enriched for genomic variants associated with mastitis resistance at 24 h post-IMI rather than milk production. While response genes in the liver showed this response at an early time point (3 h) post-IMI, whereas responsive genes at later stages were enriched for genomic variants associated with milk production. They added that all pathways activated in the liver were primarily related to the innate immune system and were persisting for up to 12 h and all the three cattle breeds shared similarities in the genetic basis of these traits.

Previous studies indicated that soon after IMI, the liver initially increases its immune response (e.g., increased production of acute phase proteins) and then decreases its overall metabolism, particularly of lipids and cholesterol [66].

Studying the genomic framework for integrating multiple layers of biological knowledge will provide novel insights into the biological basis of complex traits and improve the accuracy of genomic prediction. The SNP set

test might be used as a first-step to improve the genomic feature best linear unbiased prediction (GFBLUP) models. These approaches will become increasingly useful, as the functional annotations of genomes keep accumulating for a range of species and traits [67].

CONCLUSION

Mastitis remains one of the most economically devastating diseases in dairy cows. Vaccination is one tool that could be used to prevent mastitis. The combination of vaccination with other infection control procedures, such as excellent milking hygiene procedures, treatment of clinical cases, segregation and culling of known infected cows are important preventative measures that usually result in a significant reduction in the incidence and duration of intramammary infections. Furthermore, thehigh sensitivity of the DNA chip method can also detect trace amounts of pathogenic microorganisms that may be present before the symptoms of mastitis become evident in dairy cows. This new detection method using DNA chip technology will be useful as a highly sensitive genetic-screening method for the control of bovine mastitis. Further studies concerning incorporating biological information from gene expression data should continue to provide novel biological insights into the genetic basis of such complex traits. In addition, the SNP set test can be used as a computationally fast way to develop more predictive GFBLUP or similar models.

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