Chapter

Control and Prevention of Mastitis: Part Two

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Abstract

Current mastitis control measures are based upon good milking time hygiene; use of properly functioning milking machines; maintaining clean, dry, comfortable housing areas; segregation and culling of persistently infected animals; dry cow antibiotic therapy; proper identification and treatment of cows with clinical mastitis during lactation; establishing udder health goals; good record-keeping; regular monitoring of udder health status and periodic review of mastitis control program. Despite significant effect of these control measures when fully adopted, especially on contagious mastitis pathogens, these measures are not equally adopted by all farmers, and mastitis continues to be the most common and costly disease of dairy cattle throughout the world.

Keywords: mastitis, prevention, control, hygiene, antimicrobial, vaccine, treatment

1. Introduction

Despite significant effect of current ten points mastitis control measures when fully adopted, especially on contagious mastitis pathogens, these measures are not equally adopted by all farmers, and mastitis continues to be the most common and costly disease of dairy cattle throughout the world.

Despite decades of research to develop effective vaccines against major bacterial mastitis pathogens such as *Staphylococcus aureus*, *Streptococcus uberis*, and *E. coli*, in dairy cows, effective intramammary immune mechanism is still poorly understood, perpetuating reliance on antibiotic therapies to control mastitis in dairy cows. Dependence on antibiotics is not sustainable because of its limited efficacy and increased risk of emergence of antimicrobial-resistant bacteria that pose serious public health threats. Most vaccination strategies for prevention of mastitis have focused on the enhancement of humoral immunity. Development of vaccines that induce a protective cellular immune response in the mammary gland has not been well investigated. The ability to induce cellular immunity, especially neutrophil activation and recruitment into the mammary gland, is one of the key strategies in the control of mastitis, but the magnitude and duration of increased cellular recruitment into the mammary gland will lead to a high number of somatic cells and poor milk quality. So the sustainable control measure is to develop effective vaccines that can induce potent and effective balanced (cellular and humoral) immunity, which prevents production loses and reduces clinical severity of mastitis without stimulating a marked inflammatory response of long duration.

2. Hygienic control measures

Current mastitis control programs devised in the 1960s based on teat disinfection, antibiotic therapy, and culling of chronically infected cows have led to considerable progress in controlling contagious mastitis pathogens such as *Streptococcus agalactiae* and *Staphylococcus aureus*. However, these procedures are much less effective against environmental pathogens, particularly *Streptococcus uberis and E. coli* which accounts for a significant proportion of subclinical and clinical mastitis in lactating and nonlactating cows and heifers [1–4]. The National Mastitis Council developed a 5-point mastitis control program in 1969 to control the incidence rate of mastitis. This 5-point mastitis control program includes (1) dipping teats in an antiseptic solution before and after milking, (2) proper cleaning and maintenance of milking equipment, (3) early detection and treatment of infected animals, (4) dry cow therapy with long acting antibiotics to reduce duration of existing infection and to prevent new intramammary infection, and (5) finally culling chronically infected animals [5, 6]. Later, it was updated to a 10-point plan, which includes more steps such as establishing udder health goals, maintain clean, dry, and comfortable environment, proper milking procedures, proper maintenance and use of milking equipment, good record keeping, management of clinical mastitis during lactation, effective dry cow management including blanket dry cow therapy, maintenance of good biosecurity for contagious pathogens and marketing chronically infected cows, regular monitoring of udder health status, and periodic review of mastitis control program [7]. Though these hygienic milking practices and control measures decrease bacterial spreading, transmission, and subsequent infection, it does not fully prevent infections from establishing. Dairy farmers utilize antimicrobials as a prophylactic treatment for the prevention of mastitis or as therapeutics to treat cases of mastitis [8].

3. Use of antimicrobials for treatment and prevention of mastitis

Antibiotics are used extensively in food-producing animals to combat disease and to improve animal productivity. On dairy farms, antibiotics are used for treatment and prevention of diseases affecting dairy cows, particularly mastitis, and are often administered routinely to entire herds to prevent mastitis during the dry or non-lactating period. Use of antibiotics in food-producing animals has resulted in healthier, more productive animals; lower disease incidence and prevalence rates, reduced morbidity and mortality; and production of abundant quantities of nutritious, high-quality, and low-cost food for human consumption. In spite of these benefits, there is considerable concern from public health, food safety, and regulatory perspectives about use of antibiotics in food-producing animals [9]. There has been a growing concern with the extensive use of antimicrobials in production animals, especially non-therapeutic usage such as dry cow therapy in the case of dairy production, because of potential emergence and spread of antimicrobial resistant bacteria. There has been an increased incidence of antimicrobial resistant bacteria both in human and animal medical services.

In almost all dairy farms in the US and many other countries, intramammary infusion of long-acting antimicrobials to dairy cows at dry-off is a routine practice to prevent bacterial IMI during the dry period. Over 90% of dairy farms in the US

infuse all udder quarters of all cows with antimicrobial (blanket dry cow therapy) regardless of their health status [8, 10, 11]. Antibiotics are also heavily used in dairy farms for the treatment of cases of mastitis and other diseases of dairy cows such as metritis, endometritis, retained placenta, lameness, and pneumonia. Similarly, antibiotics are also used for the treatment of neonatal calf diarrhea and pneumonia in dairy calves. This practice exposes a large number of animals to antimicrobials and increases the use of antimicrobials in dairy farms. Antimicrobials for the treatment of mastitis are given through intramammary infusion as well as administered parenterally to dairy herd for the treatment of clinical (acute or peracute) mastitis and other periparturient diseases of dairy cows such as metritis, endometritis, retained placenta, and others like lameness and pneumonia. Antimicrobial treatment for neonatal diarrhea and pneumonia are also given through parenteral routes. Some farms also feed waste milk (discarded milk during antibiotic treatment, milk after parturition before allowed into the bulk tank) to heifer calves, which puts their gastrointestinal tract (GIT) microbiota under antibiotics pressure. Antibiotics infused into the mammary glands can be excreted to the environment through leakage of milk from the antibiotic-treated udder or absorbed into the body and enter the blood circulation and biotransformed (pharmacokinetics) in the liver or kidney and excreted from the body through urine or feces into the environments. Therefore, both parenteral and intramammary administration of antibiotics has a significant impact on other commensals or opportunistic bacteria in the gastrointestinal tract of dairy cows. This practice exposes large numbers of healthy cows to antimicrobials and also increases the use of antimicrobials in dairy farms, which in turn creates intense pressure on microbes in animals' body and farm environments.

Intramammary infection may progress to clinical or subclinical mastitis [12]. Clinically infected udder is usually treated with antimicrobial, whereas subclinically infected udder may not be diagnosed immediately and treated but remained infected and shedding bacteria through milk throughout lactation. The proportion of cure following treatment of mastitis varies and the variation in cure rate is multifactorial including cow factors (age or parity number, stage of lactation, and duration of infection, etc.), management factors (detection and diagnosis of infection and time from detection to treatment, availability of balanced nutrition, sanitation, etc.), factors related to antimicrobial use patterns (type, dose, route, frequency, and duration), and pathogen factors (type, species, number, pathogenicity or virulence, resistance to antimicrobial, etc.) [13, 14].

The most common antibiotics used to treat mastitis include cephalosporins (53.2%), followed by lincosamide (19.4%) and non-cephalosporin β-lactam antibiotics (19.1%) [8]. The problem with the use of non-selective blanket antimicrobials administration to dairy cows as a prophylactic control of mastitis is that they put selective pressure on both mastitis-causing bacteria as well as commensal bacteria in the animals' body [15, 16]. The ultimate result may not be different but the exposure level to antibiotics and its biotransformed products are different for the bacteria in the gut, in the mammary glands, and dairy farm environments during use of antimicrobials for prevention and treatment of mastitis and other diseases of dairy cattle. This selective pressure can result in antimicrobial resistant bacteria that become difficult to clear and persistent on farms and spread among animals [17]. The antimicrobial resistant bacteria or their genes may spread from these sources to human or animals or to other bacteria. McAllister et al. [18] found that CNS could potentially transfer penicillin, cephalosporins, and fluoroquinolones resistant genes to *S. aureus*. The transfer of these antibiotic resistance genes could

lead to the development of antimicrobial resistant bacteria including methicillin-resistant *S. aureus* (MRSA) [18]. Treatment of *Staphylococcus aureus* mastitis with antibiotics is of limited success which may dictate the culling of the animal [14, 19]. Until recently, MRSA was a common antimicrobial resistant strain mainly found in human hospitals; however, recent findings indicated that it has also been increasingly isolated from cattle herds [20]. The major problem with MRSA is that it is mostly resistant to multiple commonly used antimicrobials (multidrug resistant) and difficult to control and eliminate [21]. On an average, the cure rate of lactating cow therapy against *S. aureus* mastitis is about 30% or less [22]. Currently, there is no effective vaccine against bovine *S. aureus* mastitis [23], and since treatment is of limited efficacy, control of *S. aureus* mastitis focuses on prevention of contamination and spread, rather than treatment [14, 19].

Antimicrobial resistance is a growing problem in *Staphylococcus aureus* mastitis. Antimicrobial resistance helps bacteria to stay alive after treatment with antibiotics and some of the mechanisms of resistance are the presence of antimicrobial resistance genes that can spread by horizontal transfer from bacteria to bacteria by mobile genetic elements such as plasmids, phages, and pathogenicity islands [24]. This resistance can also occur through random mutations when the bacteria are under stress [25]. In the cases of mastitis, the prevalence of antimicrobial resistant bacteria seems to be increasing at least for some antimicrobials. Studies reported over 50% of isolates that cause mastitis were resistant to either beta lactam drugs or penicillin [26]. In human medicine, methicillin resistant *S. aureus* (MRSA) is a huge problem because MRSA strains are resistant to most of antibiotics making them very difficult or impossible to treat. There have also been reports of cases of bovine mastitis caused by MRSA [27–30]. Some report that these infections are due to the human strain, but others have found MRSA strains of bovine origin [21, 31]. These authors suggested that MRSA strains isolated from bovine probably gain resistance from human MRSA strain through transfer of resistance genes [32].

Waller et al. [33] evaluated the antimicrobial susceptibility of CNS and found a difference across the species on β-lactamase production. Similarly, Sawant et al. [34] found that 18% and 46 of the *S. chromogenes* and *S. epidermidis* isolates produce β-lactamase, respectively. Sampimon et al. [35] also found a 70% resistance to penicillin in *S. epidermidis*, but more importantly found that 30% of the CNS were resistant to more than one antimicrobial.

From antimicrobial resistance perspective, environmental mastitis pathogens are very important for two reasons: (1) some members of environmental mastitis pathogens are either normal microflora or opportunistic pathogens in the gastrointestinal tract of dairy cows and frequently exposed to antimicrobials directly through oral or indirectly through parenteral routes; (2) despite strain variation, some of them are highly pathogenic for human (for example, *E. coli* 0157:H7 is normal microflora in the rectum of cattle). Of significant concern is the potential for human infection by antimicrobial-resistant environmental mastitis pathogens such as extended-spectrum beta-lactam resistant *E. coli* directly through contact with carrier animal or indirectly through the food chain. Some of the Gram-negative environmental mastitis pathogens, such as *E. coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. are the greatest threat to human health due to the emergence of strains that are resistant to all or most available antimicrobials [36, 37].

In general, the antimicrobial resistance of mastitis pathogens varies with dairy farms and bacterial species within and among dairy farms [11, 38–42]. However, the antimicrobial-resistance status of human pathogenic environmental mastitis pathogens, especially the resistance status of Gram-negative environmental mastitis

pathogens in the family of *Enterobacteriaceae*, is yet to be determined. Monitoring antimicrobial resistance patterns of bacterial isolates from cases of mastitis is important for treatment decisions and proper design of mitigation measures. It also helps to determine emergence, persistence, and potential risk of the spread of antimicrobial-resistant bacteria and resistome to human, animal, and environment [17, 43]. The prudent use of antimicrobials in dairy farms reduces emergence, persistence, and spread of antimicrobial-resistant bacteria and resistome from dairy farms to human, animal, and environment.

4. Vaccines

Several vaccine studies were conducted over the years as controlled experimental and field trials. Some of the most common mastitis pathogens that have been targeted for vaccine development are *S. aureus*, *S. agalactiae, S. uberis*, and *E. coli* [44]. Most of these experimental and some commercial vaccines are bacterins which are inactivated whole organism, and some vaccines contained subunits of the organism such as surface proteins [45], toxins, or polysaccharides.

All coliform mastitis vaccine formulations use Gram-negative core antigens to produce non-specific immunity directed against endotoxin (LPS) [44]. The principle of these bacterins is based upon their ability to stimulate production of antibodies directed against common core antigens that Gram-negative bacteria share. These vaccines do not prevent new intramammary infection but significantly reduced the clinical severity of the infection [46–48]. Experimental challenge studies have demonstrated that J5 vaccines are able to reduce bacterial counts in milk and resulted in fewer and less severe clinical symptoms [47]. Vaccinated cows may become infected with Gram-negative mastitis pathogens at the same rate as control animals but have a lower rate of development of clinical mastitis [48], reduced duration of infection [46], less loss of milk production, culling, and death losses [49, 50]. The Eviracor®J5 *E. coli* vaccine (Zoetis, Kalamazoo, MI), [51, 52], as well as the UBAC® *S. uberis* vaccine (Hipra, Amir, Spain), [53] are similar to vaccination with nonspecific killed whole bacterial cells (bacterin vaccines), achieving only partial reduction in clinical severity of mastitis.

Despite several mastitis vaccine trials conducted against *S. aureus* mastitis [54–65], all field trials have either been unsuccessful or had limited success. There are two commercial vaccines for *Staphylococcus aureus* mastitis on the market, Lysigin® (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) in the United States and Startvac® (Hipra S.A, Girona, Spain) in Europe and Canada [66]. None of these vaccines confer protection in field trials as well as under controlled experimental studies [54, 58, 62, 67]. Several field trials and controlled experimental studies have been conducted testing the efficacy of Lysigin® and Startvac® and results from those studies have shown some interesting results, namely a reduced incidence, severity, and duration of mastitis in vaccinated cows compared to non-vaccinated control cows [54, 62, 68]. Contrary to these observations, other studies failed to find an effect on improving udder health or showed no difference between vaccinated and non-vaccinated control cows [66, 69]. None of these bacterin-based vaccines prevents new *S. aureus* IMI [54, 58, 62, 67]. Differences found in these studies are mainly due to methodological differences (vaccination schedule, route of vaccination, challenge model, herd size, time of lactation, etc.) in testing the efficacy of these vaccines. It is critically important to have a good infection model that mimics natural infection and a model that has 100% efficacy in causing infection. Without a good challenge model, the results from vaccine efficacy will be inaccurate.

5. Conclusions

Current mastitis control programs are based on teat disinfection, antibiotic therapy, and culling of chronically infected cows. There is no single effective vaccine against any mastitis pathogen. The physiological nature of mammary glands where induced systemic immune responses need to cross from the body into the mammary glands, the dilution of effector immune responses by large volume of milk coupled with the ability of mastitis causing bacteria to develop immune evasion mechanisms and resistance to antimicrobials makes control of mastitis very difficult. However, developing improved and effective vaccines that overcomes these constraints using these quickly advancing molecular, genomic and immunological tools is a sustainable intervention approach.

Use of antibiotics in food-producing animals does contribute to increased antimicrobial resistance in dairy cattle and farm environments. Antimicrobial resistance among dairy pathogens, particularly those bacterial strains that cause mastitis in dairy cattle, is not increasing at alarming rate. However, antimicrobial resistance among Gram-negative bacteria particularly those strains that mainly cause disease in humans are extremely high in dairy cattle and dairy farm environments. Transmission of an antimicrobial resistant mastitis pathogen and/or foodborne pathogen to humans could occur through direct contact with animal or indirectly through the food chain, if contaminated unpasteurized milk or dairy products made from contaminated raw milk is consumed, which is another very important reason why people should not consume raw milk. Likewise, resistant bacteria contaminating meat from culled dairy cows can easily transmit to humans through consumption of undercooked meat.

We emphasize and recommend the prudent use of antibiotics in dairy farms. Strategies involving prudent use of antibiotics for treatment encompass identification of the pathogen causing the infection, determining the susceptibility/resistance pattern of the pathogen to assess the most appropriate antibiotic to use for treatment, and a long enough treatment duration to ensure effective concentrations of the antibiotic to eliminate the pathogen. Alternatives to use of antibiotics for maintaining animal health and productivity based on preventative measures, such as vaccination, improved nutrition, environmental sanitation, use of teat sealants, and selection for disease resistance genetic traits together with advances in more rapid pathogen detection and characterization systems will undoubtedly play an integral role in strategies aimed at improving dairy productivity with improved safety of dairy products for human consumption.

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