# New Strategies to Prevent Mastitis

**S Pyörälä**

*Department of Clinical Veterinary Sciences, Faculty of Veterinary Medicine, University of Helsinki, Saari Unit, Saarentaus, Finland*

## Contents

Bovine mastitis remains as the disease causing the biggest economic losses to the dairy industry, despite the intensive research and prevention measures at herd level carried out for decades. Antibiotics are widely used to combat mastitis, but focus should be shifted from treatment to more economical and efficient prevention. The bacteriological aetiology of mastitis has changed from contagious to environmental pathogens, which has reduced the efficacy of the traditional mastitis control strategies. Considerable progress in the understanding of epidemiology, immunology, diagnostics and pathogenesis of mastitis has been made. The modern molecular biological methods offer good possibilities for the research of the epidemiological and virulence aspects of bacteria, which may help in building-up specific mastitis control strategies for dairy herds. Studies on the host response and relationship between somatic cell count and susceptibility to mastitis offer tools for genetic improvement of dairy cows. Biotechnological approaches for mastitis prevention are in the developmental stage, but many problems are associated, e.g. with vaccination of dairy cows against mastitis. Different methods of immunomodulation for the prevention of mastitis have shown promise in experimental trials, but the evidence is not yet enough to support commercial applications. Improving nutrition, housing and environment of dairy cattle are still crucial in the prevention of mastitis, especially during the most susceptible period after parturition. New milking techniques including robotic milking may provide better possibility for proper milking and improved udder health. Mastitis control should be part of the herd health programme in the dairy herds. In this paper, results from recent research and proposals for new prevention strategies in the field of mastitis are reviewed.

## Introduction

Despite intensive research over the decades, bovine mastitis has not disappeared and not even considerable reduction of mastitis has occurred. In the past, the biggest economic losses were because of contagious mastitis pathogens such as *Streptococcus agalactiae* and *Staphylococcus aureus* (Erskine et al. 1987; Myllys et al. 1994; Lam 1996). During the recent decades, the proportion of environmental pathogens like coliforms and environmental streptococci as mastitis causing agents has in many countries increased (Schukken et al. 1989). On the other hand, the share of the so-called minor pathogens such as coagulase-negative staphylococci and *Corynebacterium bovis* has also increased (Schukken et al. 1989; Myllys et al. 1998). Mastitis control programmes have not been fully efficient against environmental pathogens (Schukken et al. 1990; Erskine 2000). There is a growing concern among dairy producers about the economical value of extensive but inefficient antibiotic therapy of mastitis and a general concern about the possible health risks of residues in milk (Clements 1998; Van-den-Bogaard and Stobberingh 1999). The drug industry alone still believes that antibiotics lead the way in the fight against mastitis (Culloty 1998).

The ultimate control of mastitis relies on prevention rather than treatment and new strategies for this are needed.

## Defence mechanisms of the cow against mastitis

### Specific and unspecific immunity

The ruminant mammary gland has two defence mechanisms: humoral, inherited immunity and specific, acquired defence (Kehrli and Harp 2001). To the unspecific system belong the physical factors of the teat such as construction of the teat and layer of keratine. The cellular defence mechanism comprises macrophages, lymphocytes and neutrophils. Quarters with elevated somatic cell count (SCC) are more resistant to mastitis than quarters with low SCC (Burvenich et al. 2000). In the cow udder, homing of lymphocytes which is part of the acquired immunity, is shared with the peripheral immune system rather than the mucosal immune system (Kehrli and Harp 2001; Dosogne et al. 2002). Periparturient, high yielding cows are especially susceptible to mastitis and have been suggested to be immunocompromised (Burvenich et al. 2000). The possible reasons for this could be the physiological stress of lactogenesis, decreased number of circulating neutrophils capable of phagocytosis, delayed inflammatory response and impaired bacteria killing capacity of neutrophils (Detilleux et al. 1995; Burvenich et al. 2000; Meglia et al. 2001).

The antibacterial factors in the milk consist both specific and unspecific factors. Immunoglobulins form specific immunity components and they mainly penetrate into the udder during inflammation but are also formed locally (Burvenich et al. 2000; Kehrli and Harp 2001). Immunoglobulins are opsonizing agents, prevent bacterial colonization and neutralize toxins. The unspecific parts of the defence mechanism of the udder are complement, lysozyme, lactoperoxidase-tiocyanate system and lactoferrin (LF). Complement is a complex of proteins, which is present in the serum and milk and enhances phagocytosis. The concentration of complement is rich in colostrum and mastitic milk, but low in normal milk. The role of complement as a preventing factor for mastitis is probably minor (Reiter 1985).

Lysozyme is the bactericidal protein of milk, which lyses the peptidoglycan wall of Gram-positive bacteria and outer membrane of Gram-negative bacteria. Lysozyme has been shown to inhibit the growth of *Escherichia coli* in milk *in vitro*, together with complement and IgA. Lysozyme and LF potentiate the effect of each other in killing Gram-negative bacteria (Ellison and Giehl 1991). In cow's milk the concentration of lysozyme and IgA is low and their role in mastitis protection is small. Lactoperoxidase together with tiocyanate and hydrogen peroxide is bacteriostatic against Gram-positive and bactericidal against

Gram-negative bacteria. The udder produces low amounts of hydrogen peroxide and the amount of tiocyanate is related to feeding. In practice the concentration of oxygen in the mammary gland is so low, that this antibacterial system cannot work efficiently (Sordillo et al. 1997).

Lactoferrin is a glycoprotein present in milk and other secretions (Smith and Schanbacher 1977). Lactoferrin binds iron and prevents the supply of iron of bacteria. The supply of iron is the most important factor in limiting the growth of aerobic bacteria; streptococci and anaerobes have low requirement for iron and LF does not affect them (Bishop et al. 1976). It is unlikely that LF would have a strong effect during lactation, because the concentration of LF is low and the concentration of citrate high. During dry periods, LF protects the cow udder from coliform infections. Exogenic LF could be a potential substance for mastitis prevention and therapy but more research is needed (Kutila et al. 2001).

### Genetic aspects

The conformation of the cow and in particular anatomical characteristics of the udder and teats affect general resistance against mastitis and they have been used as selection criteria in breeding dairy cows for long (Erskine 2000). Selection against mastitis has specifically been targeted towards low milk SCC and is based on the heritability of SCC (Shook 1988; Emanuelson 1997). As SCC also forms a part of the defence system of the udder against mastitis, it has been suggested that with very low SCC the risk to environmental mastitis may increase (Hogan et al. 1989; Schukken et al. 1990). However it is not likely that SCC levels of dairy cows will through breeding reach 'too low' levels in the near future. Dairy cows have been bred for high milk production, and there is a positive correlation between high milk yield and mastitis (Syvajarvi et al. 1986; Fleischer et al. 2001). Direct selection on the clinical trait, mastitis, could be included in dairy cattle breeding programmes (Emanuelson 1997) and has been practised in Norway with good results (Heringstad et al. 2001b). Breeding against clinical mastitis relies on a health recording system used in the Nordic countries, where each case of disease treated by a veterinarian is registered nationwide on an individual cow base. The posterior mean of heritability of liability to clinical mastitis was 0.07 which indicates very precise interference (Heringstad et al. 2001a) but is still much lower than the heritability of SCC, 8–14% (Burvenich et al. 2000). In the breeding programmes of dairy cattle, traditional lactation period models have been used for selection basis of SCC, but an improved approach, a random regression test day model has been recently proposed (Mantysaari et al. 2000).

## Biotechnology in the prevention of mastitis
### Vaccination

Antibacterial systems are present in the mammary gland, but their efficacy against mastitis is very limited. The most common approach to improve natural immunity of dairy cows has been vaccination against mastitis

(Sordillo et al. 1997). Vaccines for different mastitis pathogens have been developed since decades ago, but mastitis has proven to be more problematic than most infectious diseases in this respect. One problem is the high number of mastitis pathogens and their heterogeneity. Immunoglobulins only enter the udder after mastitis has developed, which explains the poor efficacy of the old-type parenteral vaccines (Yancey 1999). Vaccines which trigger local production of immunoglobulins have had significant effect (Pankey et al. 1985). Most of the vaccination research has focused on the prevention of *E. coli* or *S. aureus* mastitis (Erskine 2000). The new molecular biological techniques introduced for the identification of the virulence factors of bacteria may prove useful for developing vaccines (Schuberth et al. 2001; Kaipainen et al. 2002).

A core antigen J5 vaccine to prevent coliform mastitis has been commercially available in USA for more than a decade (Smith and Hogan 1998). It reduces the incidence of coliform mastitis, but reports on its efficacy are still controversial (Tomita et al. 2000). The immunological basis of action is unknown. The immunoglobulins can act in neutralizing endotoxin and opsonize and activate complement, but are still not considered critical in cell mediated immunity. A new hypothetical mechanism of action of J5 was recently proposed by Dosogne et al. (2002), based on enhancing polymorphonuclear neutrophil (PMN) diapedesis upon intramammary infection. According to most, but not all studies, the commercial vaccine has reduced the severity of disease (Smith et al. 1999; Erskine 2000). The core antigen vaccine J5 is widely used in herd mastitis control programmes in USA. In addition, there have been attempts to develop *E. coli* vaccines using more specific antigens but they are still in an experimental phase (Lin et al. 1998).

To date, little success has been reported in developing an effective vaccine against any of the mastitis causing streptococcal species. Most of this research has been targeted towards *Streptococcus uberis*, and experimental vaccines e.g. based on the plasminogen activator of this pathogen have been tested but are still at an early phase (Finch et al. 1997; Yancey 1999). More interest has been devoted to the development of vaccine against *S. aureus*, but so far the results have not been completely successful. Potential antigens to be used for vaccination against *S. aureus* are capsules, adhesins, surface proteins and toxins (Foster 1991) and preliminary studies have shown efficacy in enhancing the immunity of the cow (Herbelin et al. 1997). *S. aureus* vaccines composed of pseudocapsule-enriched bacterins supplemented with haemolysins have been promising in experimental trials, but have not been commercialized (Yancey 1999). In the study of Sears et al. (2001), antigen-specific *S. aureus* vaccine was found to enhance intramammary treatment response towards *S. aureus* mastitis. The known commercially available old-type or new *S. aureus* vaccines have shown no effect or only limited efficacy in field conditions (Watson et al. 1996; Hoedemaker et al. 2001). There is improvement in mastitis vaccination, but a real breakthrough is still to be looked forward. One problem is the specificity: if immunity is good against a certain pathogen, it does not protect from another one (Sordillo et al. 1997; Lin et al. 1998).

### Immunomodulators

Different enhancers of the non-specific immunodefences, biological response modifiers or immunomodulators, could be useful in regulating the immunity of the cow (Mayr 1996; Blecha 2001). Several of them have been tested and the experiences from their use were recently reviewed by Zecconi (2000). These substances include the so-called paramunizers such as levamisole, *Propionibacterium acnes* and casein complexes and cytokines such as interferon inducers. Most prospective immune modulators have only been tested under experimental conditions. One recent field trial showed some promise in prevention of staphylococcal mastitis using a biological response modifier (Zecconi et al. 1999). Cytokines have been tested as immunomodulators and potential therapeutic substances for mastitis therapy since the end of the 1980s. Their use has been reviewed by Sordillo and Daley (1995). Recombinant interferon-$\gamma$ has shown positive effects in experimental *E. coli* model when given before challenge and also showed some effect in the treatment of established *S. aureus* infections, but some other studies with different cytokines failed to show significant positive effects. A few field trials have been carried out, but mostly with less than desirable results (Hogan et al. 1995). Problems with cytokines have been adverse effects, effect of some substances only if given before challenge and short-acting efficacy. The results from all studies on immunomodulators suggest that these substances may have the capacity to enhance defence of the udder, but their efficacy and practicality needs to be proven in clinical trials (Blecha 2001).

### Other biotechnological approaches

One way to improve resistance would be to make the mammary gland produce foreign protective proteins through transgenesis (Gordon et al. 1987). This technique has been used mainly for making cow udder as bioreactor, which would produce human proteins for the drug industry (Houdebine 1994; Wall et al. 1997). Another possibility would be to use transgenesis for cow's benefit, i.e. to add resistance to mastitis by enhancing the intrinsic antibacterial mechanisms or by making the udder to produce new antibacterial proteins or peptides. A technique has been tested in mice with a gene coding antistaphylococcal peptide lysostaphin. Lysostaphin or peptidoglycan hydrolase can destroy staphylococcal wall and kill the bacteria (Daley and Oldham 1992). Transgenic mice were significantly more resistant against mastitis as compared with normal mice in *S. aureus* challenge studies. Mice expressing the highest lysostaphin concentration in milk were totally resistant (Bramley et al. 2001). Lysostaphin has a somewhat narrow spectrum because it does not protect against environmental pathogens. According to Finnish experiences, cows expressing human LF in their milk had very low SCC in their milk and seldom had mastitis (Vesa Rainio 1999, oral communication). It may be unrealistic that mastitis would be controlled through breeding transgenic dairy cows in the near future. The reaction of the consumers towards the use of these techniques may be very critical.

## Udder health at herd level

### Environmental factors and management

Housing and environment of dairy cows have a major role in the prevention of mastitis. Cow welfare and comfort are key issues for animal and udder health (Anderson 2000) and the most critical is the periparturient period. Traditionally, contagious mastitis has been the problem of small dairy units with stanchion barns (Myllys et al. 1998) and environmental mastitis has been connected with larger units with free stall system (Schukken et al. 1989; Shpigel et al. 1998). The new molecular biological methods such as DNA fingerprinting, pulse field electrophoresis or ribotyping for the identification of bacteria offer good possibilities to study the epidemiology of mastitis (Yancey 1999; Douglas et al. 2000). In the future these techniques may be used even at the herd level, which may help in building-up specific mastitis control strategies for the herds.

The effects of management and housing are complex and the interdisciplinary team approach should be used on farms to solve problems. The use of diagnostic tests and good record keeping of clinical cases and treatments facilitates the targeting of mastitis control measures against specific pathogens. Each herd should have a comprehensive health management programme which should pay special attention to the control of udder health (Bickert and Radostits 2000).

### Milking

Milking machine contributes to udder health and mastitis. Modern milking equipments are well functioning compared with those some decades ago, but more improvements are needed. Defects in the design of milking parlour or milking equipment can result in problems such as overmilking and increase in the incidence of mastitis (Erskine 2000). Teat duct colonization and new infection risk are significantly linked with machine-induced changes in teat thickness after milking (Hamann 1995). Cows and heifers may require different types of milking units and vacuum conditions vary between clusters and liners, which may affect udder health. Proper stimulation of the teats and the udder activate milk letdown before attachment of the machine. Preparation time long enough is important to prevent unnecessary stress to the teats (Rasmussen et al. 1992). A monitoring system to determine teat end condition of dairy cows has been developed, which facilitates evaluation of the effects of milking on udder health (Neijenhuis 1998).

Automatic milking systems (AMS) are becoming more popular and may have a considerable effect on the udder health in the herd (Hamann 1999). In the AMS, cows are milked more frequently than in the conventional systems, which has a positive effect on udder health (Hillerton and Winter 1992; Rasmussen et al. 2001). The biggest problems with robotic milking are poor detection of clinical mastitis and separation of abnormal milk from the supply (Klungel et al. 2000; Rasmussen 2000). Manufacturers of the AMS use different approaches to detect mastitis, but so far none

of them has proven to be accurate enough (Hovinen et al. 2002). A self-monitoring system should be introduced for herds with AMS to maintain milk quality and udder health (Justesen 2002).

Post-milking teat dipping is considered a very important component of good milking management and is practised in most dairy herds. A correctly used teat dip can reduce the incidence of new udder infections at least by 50% (Pankey 1984). There is an abundance of products in the market with different active substances and each dip should be evaluated for its safety, efficacy, advantages and disadvantages (Erskine 2000). Pre-milking teat disinfection with iodine-based preparations is a common practice in North America. The use of pre-dipping is expected to reduce contagious mastitis but according to the few published studies, it merely has affected the incidence of environmental infections (Galton et al. 1988). It seems that more evidence is needed to assess the real benefit from pre-dipping in different herd conditions. In the European Union pre-dipping is not allowed because of the risk for residues in the milk.

### Nutrition

Nutrition is an important factor in the resistance against disease, and deficiency of some trace substances and vitamins such as selenium, copper, zinc and vitamin E have been found to be predisposing factors for mastitis (Sordillo et al. 1997). Selenium forms part of the enzyme glutathioneperoxidase, which protects the cell from reactive oxygen products. Lack of selenium suppresses phagocytosis and supplementing cows with selenium and vitamin E has had a positive effect on udder health in cows with low levels of these substances (Smith et al. 1984). Copper-supplemented heifers had lower bacterial counts, SCC and milder clinical signs in experimental *E. coli* challenge as compared with unsupplemented heifers (Scaletti et al. 2001). The concentrations of vitamin A and E, and of trace element Zn have been shown to significantly decrease at calving, which may have negative implications for the immune defence of the cow (Meglia et al. 2001). The current dietary recommendations may be insufficient for optimal immune function and response of high yielding dairy cows especially around parturition.

### Prophylactic treatment of dry cows

Antibiotic treatment of dairy cows at drying-off has been successfully practised for decades, and blanket dry cow therapy forms one of the cornerstones in mastitis control in many countries. Total dry cow therapy has been a critical part of contagious mastitis control programmes. Recently, the routine of treating all cows has been questioned. The major reasons for the selective therapy i.e. treating infected cows only would be reducing the expenses of treatments, avoiding the elimination of minor pathogens which may make the cow more susceptible for environmental pathogens, and prevent the possible emergence of resistant bacteria (Erskine 2000). Selective dry cow therapy has proven to be an efficient method for maintaining udder health and has been suggested to be the method of choice for dry

cow management (Oesteras et al. 1999). A prerequisite for selective dry cow therapy is an efficient method to identify infected cows and cows at risk. Several choices are already available for cow monitoring on the basis of SCC or other inflammation parameters and recording of clinical mastitis data (Erskine 2000). It is not advisable to go too far in selection: treatment of infected quarters only led to a greater risk of new infections (Hassan et al. 1999). Dry cow therapy has traditionally been targeted towards Gram-positive bacteria, but recent studies suggest that in some conditions protection against Gram-negative agents may also be necessary (Bradley and Green 2001). Bacteriological sampling of each case of mastitis provides data for planning dry cow therapy and other control measures in the herd.

A non-antibiotic method for dry cow protection is bismuth subnitrate based teat seal, which has shown the same efficacy than antibiotic treatment against environmental mastitis caused by streptococci (Woolford et al. 1998). A non-antibiotic antibacterial substance, lacticin 3142, was compared with teat seal alone in experimental challenge model and was found to produce significantly better protection (Ryan et al. 1999). Commercial teat seal products are not yet widely available and more research is needed to prove their efficacy and safety in dry cow management.

## Conclusions

Mastitis results from failure in the complex relationship between three factors: host resistance, mastitis causing bacteria and the environment. The most susceptible population for mastitis are the high yielding, periparturient dairy cows. Remarkable progress in the prevention of mastitis has been reached mainly in combating the contagious pathogens *S. aureus* and *Str. agalactiae*. Mastitis caused by environmental organisms and so-called minor pathogens have become the main challenge in the modern dairy industry. It seems that the conventional means to prevent mastitis may be inefficient in preventing infections caused by these bacteria. Research is needed about the host defence mechanisms to understand factors affecting susceptibility to udder infections. Breeding towards high production and low SCCs may contain a risk to decreased mastitis resistance. Biotechnology may offer promise in enhancing host defence mechanisms, but more studies are needed for developing clinical applications with proven efficacy and safety. Use of antibiotics and disinfectants have their role in mastitis control but we cannot rely solely on them.

Probably, the best progress in the control of mastitis in the short term can be made by improving the nutrition, environment and management of dairy cows. The housing of the cows should allow them to be clean, dry and comfortable, and free from stress. Negative energy and protein balances after parturition may disturb the immune system and should be avoided. New milking techniques including AMS may provide a possibility for better milking and improved udder health if used properly. In each herd, mastitis control should be part of the herd health programme.

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Authors' address (for correspondence): S. Pyörälä, Department of Clinical Veterinary Sciences, Faculty of Veterinary Medicine, University of Helsinki, Saari Unit, FIN-04920 Saarentaus, Finland.
E-mail: satu.pyoraia@helsinki.fi