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# Mastitis in Post-Partum Dairy Cows

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Transition from the dry period to lactation is a high risk period for the modern dairy cow. The biggest challenge at that time is mastitis. Environmental bacteria are the most problematic pathogens around parturition. Coliforms are able to cause severe infections in multiparous cows, and heifers are likely to be infected with coagulase-negative staphylococci. During the periparturient period, hormonal and other factors make the dairy cows more or less immunocompromised. A successful mastitis control programme is focused on the management of dry and calving cows and heifers. Clean and comfortable environment, proper feeding and adequate supplementation of the diet with vitamins and trace elements are essential for maintaining good udder health. Strategies which would enhance closure of the teat canal in the beginning of the dry period and would protect teat end from bacteria until the keratin plug has formed decrease the risk for mastitis after calving. Dry cow therapy has been used with considerable success. Yet, a selective approach could be recommended rather than blanket therapy. Non-antibiotic approaches can be useful tools to prevent new infections during the dry period, in herds where the risk for environmental mastitis is high. Vaccination has been suggested as a means to support the immune defence of the dairy cow around parturition. In some countries, implementation of *Escherichia coli* core antigen vaccine has reduced the incidence of severe coliform mastitis after calving.

## Introduction

Rate of bovine intramammary infections (IMI) is at the highest level around parturition (Fig. 1) (Smith et al. 1985; Bradley and Green 2004; Valde et al. 2004; McDougall et al. 2007). The factors influencing the susceptibility of the mammary gland to infections are presence of bacteria at the teat end, level of efficacy of the protective characteristics of the teat canal and defence mechanisms in the udder (Sordillo 2005). During the dry period, the mammary gland is considered to be very resistant against infections. The streak canal is closed and sealed by a keratin plug. In the fully involuted gland, the concentrations of many soluble factors are at a high level and effective in preventing new infections. Concentration of leucocytes is high in the dry gland, and the environment is more favourable for their function than in the lactating gland (Burvenich et al. 2007). The recent decades have shown considerable progress in the understanding of the function of the defence system of the bovine mammary gland. Despite this, the inefficient host defence and increased susceptibility to mastitis during the transition period continue to be the major problems in the dairy cows (Leslie and Dingwell 2002). In this article, different factors affecting the susceptibility of the cow to mastitis around parturition, as well as measures for prevention of mastitis, are reviewed.

## The Effect of the Dry Period

During involution and again towards the end of the dry period, the risk for mastitis is at the highest (Oliver and Sordillo 1988). After drying-off, milk is no longer removed from the udder, and intramammary pressure may cause leakage of milk from the teats. Leucocytes start entering the gland within 1 week after dry-off, but do not immediately protect the gland. The keratin plug, which also contains inhibitory substances against bacteria, is formed within 1–2 weeks after dry-off and should naturally seal the teat (Dingwell et al. 2004). Quarters that form a keratin plug, which completely closes the teat soon after dry-off, have significantly less risk to develop an IMI. Yet, as many as 23% of the quarters have been found to be still open 6 weeks after drying-off (Dingwell et al. 2004). In an earlier study, 5% of the teats were found to remain completely open (Williamson et al. 1995). Increasing milk yield at the dry-off has been recognized as a significant risk factor: every 5 kg increase in milk yield at dry-off above 12.5 kg increased the odds of the cow by 77% to have an IMI caused by environmental bacteria at calving (Rajala-Schultz et al. 2005). The increased susceptibility to mastitis with increasing milk yield at drying-off is probably related to the incomplete closing of the teat canal (Dingwell et al. 2004). Teat end condition also affects mastitis susceptibility: teats with cracked ends have higher odds of developing IMI around calving (Dingwell et al. 2004).

## Mammary Gland Immunity Around Parturition

From 2 weeks prior to calving, until about 2–3 weeks after calving, is the most critical period for the health of the mammary gland (Oliver and Sordillo 1988). The innate immune system of the periparturient cows is compromised. During colostrogenesis, the susceptibility of the mammary gland to infections increases as the teat canal starts to open and leaks mammary secretion (Oliver and Sordillo 1988). At the same time, the protective effect of dry cow therapy (DCT), if used, has disappeared (Oliver et al. 1990). Hormonal changes include steep rise of the concentration of 17$\beta$-oestradiol in the plasma during the last week of gestation, peaking during the last days before parturition and a simultaneous drop of progesterone in circulation. Blood cortisol increases about fivefold at the day of parturition (Burton et al. 2005).

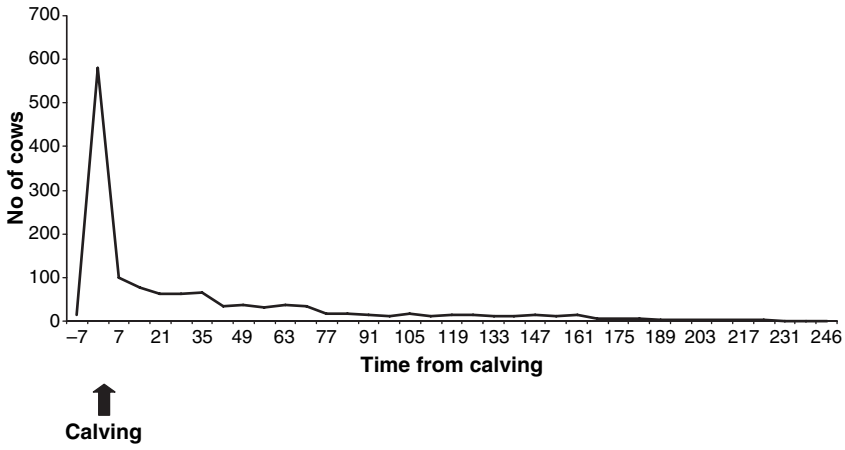


Fig. 1. Distribution of the time from calving (days in milk) to the diagnosis of clinical mastitis, based on data from 28 dairy herds in New Zealand (McDougall et al. 2007)

## Cellular Factors

Polymorphonuclear neutrophils (PMN) belong to the innate immune response of the mammary gland to infections (Paape et al. 2003; Burvenich et al. 2007). Around parturition, many functions of the PMN are impaired. The number of immature neutrophils in circulation increases and the number of mature neutrophils in the blood and milk are at the lowest. The production of reactive oxygen species (ROS) to kill bacteria is reduced from 1 week before parturition over the first 2 weeks after calving (Hoeben et al. 2000; Mehrzad et al. 2002). The change in the respiratory burst activity has been found to be parallel with peripartum increase of concentrations of 3$\beta$-hydroxybutyric acid (3-BHB), bovine pregnancy-associated glycoprotein and bilirubin (Hoeben et al. 2000). The rapid rise of the concentration of blood cortisol induces changes in the function of PMN, supporting their extended life span in the blood and increasing release from the bone marrow. At that time, neutrophils favour tissue remodelling over defence against infections as their primary task (Burton et al. 2005). At parturition, large numbers of leucocytes are recruited to the reproductive tract and placenta. The ability of the cells to marginate on and migrate through endothelium to infected peripheral tissue in other sites than uterus decreases. After parturition, cortisol down-regulates its own receptors in neutrophils and the system returns to normal function (Burton et al. 2005). The effect of steroid hormones on bovine PMN function was studied by Lamote et al. (2004), who showed that 17$\beta$-oestradiol treatment decreased the number of viable cells but progesterone had no effect. In periparturient cows, a loss of expression of critical neutrophil adhesion molecules has been seen (Monfardini et al. 2002), and this loss has been associated with the elevated cortisol levels (Weber et al. 2004). The same was demonstrated by external glucocorticoid administration (Burton et al. 2005). The proportion of PMN expressing, for example, the adhesion receptor L-selectin, which is necessary for penetration to the sites of infection, is diminished (Diez-Fraile et al. 2004). Neutrophil extracellular traps have recently shown to have a role in killing of bacteria and also to be fully capable to function in the milk environment. The impaired efficiency of this system during the periparturient period may be one more explanation for the immunosuppression of the dairy cows at that time (Lippolis et al. 2006).

Lymphocytes can recognize antigens through specific receptors and are divided to T and B lymphocytes. CD4+ T lymphocytes cells activate lymphocytes or macrophages to secrete cytokines, which then can facilitate either cell-mediated or humoral immune response (Sordillo 2005; Rainard and Riollet 2006). The proportion of CD4+ cells in blood and mammary gland declines post-partum, and their cytokine production is different from that in mid-lactating cows. In periparturient cows, the percentage of T cells has been shown to substantially decline from that in mid-lactating cows (Shafer-Weaver et al. 1996). Macrophages are the dominant cell type in milk of healthy, lactating gland. During infection, macrophages initiate the immune response by releasing cytokines and other substances augmenting local inflammatory process (Rainard and Riollet 2006). Bovine macrophage numbers are highest in the mammary gland during the last week of gestation, but their phagocytic capacity is decreased (Sordillo 2005).

## Humoral Factors

Innate and specific soluble factors represent an important part of the defence in the mammary gland; complement, lactoferrin, lysozyme and antimicrobial peptides are the most common (Rainard and Riollet 2006). Lactoferrin is most active during the steady state of involution (Smith and Schanbacher 1977). During that time, lactoferrin efficiently prevents growth of bacteria with a high demand of iron such as coliforms (Todhunter et al. 1991). Complement is present in high concentrations in colostrum, and seems not to be a limiting factor in the defence of the mammary gland at that time (Rainard 2003). The most important factors of the specific immune response are opsonizing immunoglobulins (Ig) produced by antigen-activated B lymphocytes. $IgG_1$ is the primary isotype present in the healthy mammary gland, but $IgG_2$ increases during inflammation. The concentration of IgGs in the bovine serum is lower around parturition and in particular, the lack of

the $IgG_2$ isotype is associated with the increased indicence of mastitis (Mallard et al. 1998).

## Dry Cow Therapy

Use of DCT has an impact on the incidence of puerperal mastitis in two ways: first DCT should eliminate infections present in the mammary gland at dry-off and thus prevent their flare-ups at calving; the second aim is to protect the mammary gland for new IMIs during the dry period (Robert et al. 2006). Antimicrobial substances used for DCT do not generally persist in the udder until calving and thus do not offer protection at that time (Oliver et al. 1990). Yet, in an UK study, reduction of clinical mastitis caused by Gram-negative agents in the subsequent lactation was demonstrated with a long-acting intramammary preparation with Gram-negative spectrum (Bradley and Green 2001). These are the findings to date to demonstrate that selection of dry cow treatment can influence the incidence of clinical mastitis in the subsequent lactation.

During the dry period, 8–12% of previously healthy quarters develop IMI which can be detected at calving if no DCT is administered (Leslie and Dingwell 2003). New IMIs which occur around parturition may greatly impact production in the subsequent lactation (Oliver and Sordillo 1988; Whist et al. 2006). Blanket DCT continues to be the standard procedure in most countries (Leslie and Dingwell 2003). Routine treatment of all cows has recently been questioned, since bulk milk somatic cell counts (SCC) have markedly decreased and mastitis has changed from contagious to environmental (Leslie and Dingwell 2003). In some countries, especially in Scandinavia, blanket DCT has never been adapted but selective DCT therapy recommended (Osteras et al. 1999; Robert et al. 2006). Dry cow therapy does not necessarily protect cows from mastitis as according to a Canadian study, as much as 11% of the treated cows had new IMI after the dry period (Dingwell et al. 2004). Pre-partum intramammary antibiotic therapy for heifers has been suggested to reduce CNS mastitis during first lactation (Oliver et al. 2003; Middleton et al. 2005). In a recent study (Borm et al. 2006), no advantage from this practice could be shown. Non-antibiotic internal teat sealants have become widely used, and have proven to be effective in prevention of new infections during the dry period (Huxley et al. 2002).

## Effect of the Metabolic State

The most important metabolic disturbances occurring shortly after calving are milk fever, ketosis and abomasal displacement. Hypocalcaemia affects the digestive system and pre-disposes the cow to concomitant diseases. It may affect the teat end sphincter and thus increase the risk for mastitis. Cows with periparturient hypocalcaemia are reported to have greater chance of developing coliform mastitis (Curtis et al. 1983). Negative energy balance and perhaps protein imbalances in early lactation contribute to the impaired immune defence (Spain and Scheer 2006). Disturbance in fat metabolism and severe negative energy balance may lead to fatty liver and ketosis. Accumulation of fat in the liver disturbs production of humoral immune factors and is also associated with decreased functional capacity of PMN (Zerbe et al. 2000). There is evidence for a decreased capacity for phagocytosis and killing of bacteria in cows suffering from ketosis and fatty liver (Leslie et al. 2001). High concentrations of ketone bodies such as BHB and acetoacetate found at parturition have been shown to inhibit the proliferation of haematopoietic cells (Hoeben et al. 2000). Elevated levels of BHB were associated with increased incidence of clinical mastitis during early lactation (Smith et al. 1985; Huszenicza et al. 2004). The course of mastitis was severe in all ketotic cows regardless of the chemotactic response before infection (Kremer et al. 1993b).

## Role of the Causing Agent

Bacteriological aetiology of mastitis during the puerperal period differs between countries, as well as between heifers and older cows. The biggest bacterial challenge for the bovine udder at parturition comes from the environment of the cow (Oliver and Sordillo 1988). Coliform bacteria appear to be a major problem for the periparturient cow (Burvenich et al. 2007). In the UK, a high proportion of puerperal mastitis is caused by coliform bacteria, mainly *Escherichia coli* (Bradley and Green 2004). In a Canadian study, the prevalence of IMIs at freshening was 34% and most new IMIs were caused by environmental streptococci and coliform bacteria (Dingwell et al. 2004). Mastitis caused by *Streptococcus uberis* is most common during the dry period and in early lactation, and in some countries the pathogen is most frequently isolated after calving (Smith et al. 1985; McDougall et al. 2007). On the contrary to environmental pathogens, the incidence of mastitis caused by the contagious pathogen *Staphylococcus aureus* has been found to increase towards later lactation (Sol et al. 2000; McDougall et al. 2007). In Norwegian small herds mostly kept in tie stalls, the pathogen most commonly isolated at calving from cows in the first lactation was *S. aureus* (Waage et al. 1999). In many countries, the most commonly isolated organism in primiparous cows around parturition is CNS (Matthews et al. 1992; Myllys 1995; Taponen et al. 2006, 2007; Parker et al. 2007). CNS mastitis is usually mild (Taponen et al. 2006). Inflammatory reaction in the udder in mastitis caused by CNS was found to be stronger in early lactating cows as compared with later lactation (Pyörälä and Pyörälä 1998).

## The Special Problem of Puerperal *E. coli* Mastitis

Much research has been carried out on *E. coli* mastitis, which in early lactating cow is often associated with severe clinical signs (Vandeputte-Van Messom et al. 1993; Burvenich et al. 2003). Pathophysiology of coliform and staphylococcal mastitis has been shown to be different, and these pathogens elicit a different type of immune response (Bannerman et al. 2004). A strong cytokine response and acute or peracute course of the disease is typical for *E. coli* mastitis in early lactation (Burvenich et al. 2007). Inflammatory reaction of the

mammary gland to endotoxin was shown to be significantly more severe in cows in early lactation than in the same cows close to drying-off (Lehtolainen et al. 2003). The greater severity of coliform mastitis after calving has been explained by the dysfunction of PMN (Vandeputte-Van Messom et al. 1993). The number of circulating PMN has been shown to correlate with the severity of *E. coli* mastitis (Kremer et al. 1993a). Acute phase mediators produced during *E. coli* mastitis can trigger an extensive ROS production which damages host tissues; cells from periparturient dairy cattle have been shown to produce significantly more TNF-α than cells from mid-lactating cows (Sordillo et al. 1995). Multiparous cows have shown to develop more severe *E. coli* mastitis (Vangroenweghe et al. 2004) as compared with young cows. Blood PMN function seems to be more efficient in young cows than in older cows (Burvenich et al. 2003). The viability and production of ROS of the milk neutrophils to kill bacteria was found to be depressed in multiparous cows (Mehrzad et al. 2002).

In the UK, studies have shown that enterobacteria are able to infect the udder during the dry period and persist there until parturition (Bradley and Green 2000). This has not been confirmed in other countries. For example, in a recent US study, the proportion of coliforms as mastitis causing agents was less than 1% at drying-off and after calving (Pantoja and Ruegg 2007). In the study by Smith et al. (1985), a relatively high proportion of udder infections at the beginning and at the end of the dry period was found to be due to Gram-negative bacteria. Dried manure with high counts of coliform bacteria was used as bedding, which may have affected the results.

## Diagnosis of Mastitis After Calving

Udder health of a dairy cow should be assessed as soon as possible after calving. Diagnosis of clinical mastitis in early lactation must be adjusted according to the physical properties of milk during that period. Somatic cell count is increased at parturition, but decreases to normal levels within 3–4 days. The difference between infected and healthy quarters is significant at both times (Barkema et al. 1999a). California mastitis test (CMT) was found to have sensitivity and specificity high enough for detecting IMI caused by major pathogens at day 3 post-partum (Sargeant et al. 2001). Regular monitoring of the udder of the cows around calving is most important, and milk should be examined as soon as possible after calving (Green et al. 2007).

## Effect of Dry Cow Management on the Occurrence of Mastitis Post-Partum

Dry cow management, such as environment, feeding and timing of transfer to the calving unit, significantly affect the susceptibility of heifers or dairy cows to puerperal mastitis. Generally, the incidence of clinical mastitis after calving increases with parity (Whist et al. 2006; Green et al. 2007). Dry and transition diets should contain the recommended levels of vitamins and trace elements (Spain and Scheer 2006). The most critical are vitamin E and selenium, but also vitamin A, copper and zinc have a role in the defence of the host against infections (Godden et al. 2006). Decades ago, supplementation of dairy cows with selenium and vitamin E was shown to have a positive effect on udder health (Smith et al. 1984; Hogan et al. 1993). The effect has mostly been seen in herds fed with deficient or low levels of these elements. In herds fed with normal diets, daily vitamin E supplementation during the puerperal period did not affect the incidence of clinical mastitis or other puerperal diseases (Persson et al. 2007).

Dry matter intake should not be restricted during the dry and transition period, but overfeeding of dry cows should be avoided. Regular body condition scoring is a good tool in monitoring the efficiency of feeding. In a recent study, it was associated with less risk of clinical mastitis (Green et al. 2007). In a Swedish study, factors which increased the risk of elevated SCC after calving were intensive concentrate feeding to heifers, and moving to confined housing on the day of calving instead of earlier (Svensson et al. 2006). The results from an Estonian study agreed with the Swedish results, as moving heifers from separate housing to the tie stall less than 2 weeks before parturition significantly increased the risk for clinical mastitis around parturition (Kalmus et al. 2007). An increasing risk of clinical mastitis was found in herds which housed heifers with older cows (Barkema et al. 1999b). In pasture-grazed conditions, risk factors for peripartum mastitis may differ from those in housed herds (Compton et al. 2007). Significant risk factors for clinical and subclinical mastitis post-calving were pre-calving subclinical mastitis, low teat height above the ground, Friesian breed and udder edema.

Providing a clean, dry environment, good cow comfort and ventilation are extremely important in prevention of mastitis in dry and calving cows and heifers. A recent study from the UK demonstrated the importance of these factors in protecting cows from clinical mastitis after calving (Green et al. 2007). Good drainage of the dry cow accommodation, use of mattresses on dry cow cubicle surfaces and disinfection of cubicle beds were some of the factors found to be protective against mastitis (Fig. 2). Thickness of bedding of the calving box was found to be negatively correlated with incidence rate of clinical mastitis (Barkema et al. 1999b). In a Belgian study (De Vliegher et al. 2004) in herds with heifers calving on slatted floors, heifers had lower SCC, probably because those calving places were cleaner than premises with non-slatted floors. If possible, dry cows and pregnant heifers should not be housed in the same barn, nor the transition cows together with the milking cows (Barkema et al. 1999b; Green et al. 2007).

Development of vaccines against mastitis has been difficult, because even natural intramammary infection does not provide protection against subsequent infections (Talbot and Lacasse 2005). The high number of mastitis pathogens further complicates the task. Commercial common core antigen vaccines against coliform mastitis have been available in some countries for years. These so-called J5 vaccines are based on the whole

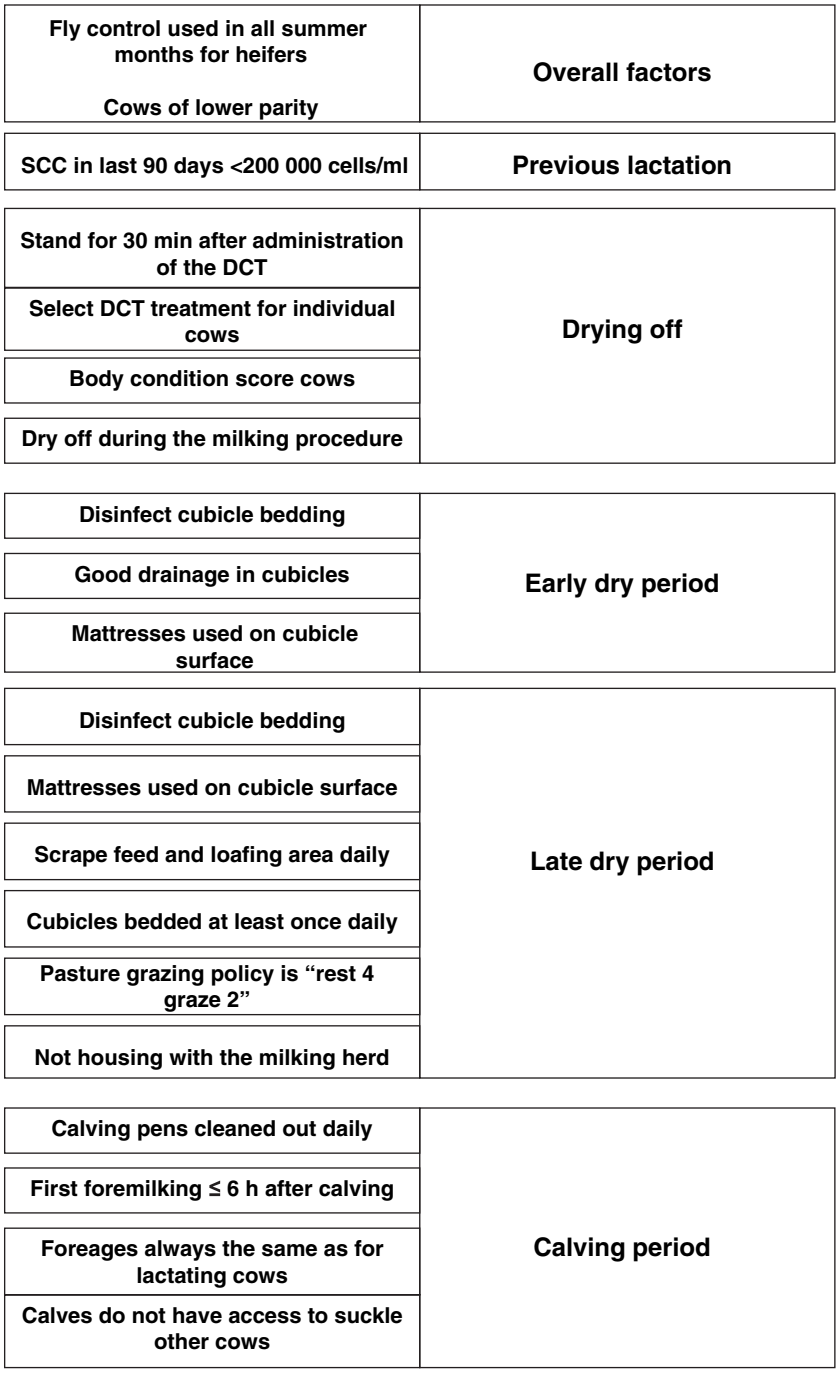


Fig. 2. Cow, farm and management factors associated with significantly reduced risk of clinical mastitis. Rest 4 graze 2 = a pasture rotation method in which dry cows graze the pasture for a maximum of 2 weeks followed by stock not grazing the pasture for a minimum of 4 weeks. Reproduced from Green et al. (2007) with permission

antigen from a mutant strain O111:B4 of *E. coli*. Vaccination produces a rather non-specific immune response against Gram-negative bacteria. The exact mechanism of action of this vaccine is not known (Dosogne et al. 2002). Incidence and severity of clinical signs of coliform mastitis have been reported to be reduced with the *E. coli* core antigen vaccine but not all studies have indicated similar efficacy (Tomita et al. 2000; Wilson et al. 2007a,b). Vaccination with J5 type vaccines only results in a short-term response and requires repeated injections, which is laborious. The efficacy of vaccines against mastitis caused by Gram-positive organisms has been even less successful (Talbot and Lacasse 2005). Development of mastitis vaccines may benefit from new molecular techniques and progress in this field may be seen in the future.

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