Somatic cell count patterns

Improvement of udder health by genetics and management

Yvette de Haas

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Somatic cell count patterns:
Improvement of udder health by genetics and management

Celgetalpatronen:
Verbetering van uiergezondheid door genetica en management

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Abstract

Clinical mastitis (CM) is one of the major diseases in dairy herds. It induces economic costs, mainly consisting of discarded milk, increased health care costs and reduced milk quality. Mastitis also contributes to consumer concerns regarding animal welfare and regarding the impact of use of antibiotics in animals on efficacy of antibiotics for human health and the possible development of antibiotic resistant bacteria. Decreasing the incidence of CM is thus of great interest of the farmer, the cow and the consumer, and could be achieved by either designing mastitis control programs, as these provide guide-lines for udder health management, or by genetic selection. Although genetic selection is a slow process, it results in a steady change in the genetic composition of the dairy herd. Research described in this thesis provide insight in the use of patterns of peaks in somatic cell count (SCC) in genetic selection and mastitis control programs. Patterns of peaks in SCC were defined based on SCC recorded on consecutive test-day, and are based on biological understanding of pathogens and of the immune system of the cow. Results showed that selecting for lower lactation-average SCC caused a shift in the importance of the main mastitis-causing pathogen. Genetic selection against occurrence of SCC patterns, however, was more effective to decrease the natural susceptibility to mastitis-causing pathogens, than selection for lower lactation-average SCC. Patterns of peaks in SCC are proven to be useful as basic tools for health management advice, as they can distinguish between cases of CM associated with either environmental or contagious pathogens, whereas the currently used primary traits were indicative for contagious, but not for environmental mastitis.

Keywords Pathogen-specific clinical mastitis, Somatic cell count patterns, Genetic selection programs, Udder health management

De koe

Een koe is een merkwaardig beest
wat er ook in haar geest moge zijn
haar laatste woord is altijd boe

(K. Schippers)

Contents

Chapter 1

General introduction

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Over the past thirty-five years, milk production of dairy cows has dramatically increased in Western Europe and North America. An increased milk production means an increased profitability for farmers. However, several studies have demonstrated that high-producing cows are at an increased risk of infectious diseases (Uribe et al., 1995; Van Dorp et al., 1998). Among these diseases, mastitis is one of the major diseases in dairy herds. Mastitis is an inflammation of one or more quarters of the udder of a cow, generally caused by bacteria. It induces economic costs, mainly consisting of discarded milk, increased health care costs and reduced milk quality (Timms and Schultz, 1984; Erb et al., 1985; Houben et al., 1993; Miller et al., 1993; Allore and Erb, 1998). Mastitis also contributes to consumer concerns regarding animal welfare (Willeberg, 1994) and concerns regarding the impact of use of antibiotics in animals on efficacy of antibiotics for human health.

Mastitis and Pathogens

Clinical *vs.* Subclinical mastitis. Visible signs of an inflammatory response of the udder characterise clinical mastitis (CM). Examples of these visible signs are abnormal texture and discoloration of the milk, swelling and discoloration of the udder, increased temperature or pain of the quarter. When signs are not clinically visible, pathogen presence has resulted in subclinical mastitis (SCM). Laboratory techniques such as bacteriological culture and measurement of somatic cell count (SCC) are then needed to detect infection and inflammation. However, bacteriological cultures can be negative when the concentration of udder pathogens is low, but observing a change in cow's cell count from under to over 200,000 cells/ml predicts inflammations (Dohoo and Leslie, 1991; Schepers et al., 1997).

Mastitis-causing pathogens. Over 100 different micro-organisms can cause mastitis, but most of the economic losses are associated with species of the coliform bacteria, the staphylococci, and the streptococci (Smith and Hogan, 2001). Mastitis-causing pathogens can be categorised depending on their aetiology into environmental and contagious pathogens (Fox and Gay, 1993; Smith and Hogan, 1993).

The primary reservoir of coliform bacteria is the environment of the dairy cow; i.e. the cubicles and bedding. *Escherichia coli* is the most important environmental pathogen in this category (Smith and Hogan, 1993). Exposure to environmental pathogens may occur at any time during lifetime, and it is independent of the presence of infections in herd mates (Zadoks et al., 2001a). Because pathogens are present in the environment of the cow, eradication of the disease through prevention of cow-to-cow transmission or elimination of reservoirs of pathogens is not possible. Several variables may be associated with the

exposure to environmental pathogens. For example, cubicle cleanliness is often associated with a lower incidence of environmental exposure, as well as the use of rubber masts in the calving areas because of a better possibility to clean them (Schukken et al., 1990).

Staphylococcus aureus is the most important contagious pathogen (Fox and Gay, 1993), and its primary reservoir is the infected animal or udder quarter, and transmission is largely limited to the milking process. Intramammary infections (IMI) with contagious pathogens are transmitted from cow to cow. For contagious pathogens, the number of new infections in a population depends on the number of infected individuals that is already present (Lam et al., 1996). In contrast with environmental pathogens, eradication of contagious pathogens is possible when reservoirs of infection are eliminated and routes of cow to cow transmissions are cut off. Middleton et al. (2002) tested the hypothesis that there are differences in pathogenicity between strains of *Staph. aureus* that cause bovine mastitis. No significant differences were found between strains, suggesting that other factors than strain-type affect the severity of the case of mastitis.

The way *Streptococcus uberis* and *Streptococcus dysgalactiae* spread is not completely clear. *Streptococcus dysgalactiae* is usually referred to as contagious (Hillerton et al., 1995), but most other streptococci are considered to be environmental (Smith et al., 1985; Pankey et al., 1987). However, strain typing studies and biometrical analyses implicated contagious transmission via the milking machine as most plausible explanation for the outbreak of *Strep. uberis* mastitis reported by Zadoks et al., (2001b) and Zadoks et al. (2003). Therefore, epidemiology of mastitis-causing pathogens is better represented by a sliding scale, where the balance of contagious and environmental transmission shifts gradually, than by a species-based dichotomy (Zadoks, 2002), and classifying mastitis-causing pathogens as purely environmental or contagious is often an oversimplification.

Minor pathogens, like coagulase-negative staphylococci (CNS) and *Corynebacterium bovis* are categorised to be contagious. An interesting observation is that minor pathogens seem to have a protective effect against infections with major pathogens (Lam et al., 1997b). From within-cow comparison, it appeared that in quarters infected with minor pathogens, infection with major pathogens was significantly lower than in comparable control quarters not infected with minor pathogens. Quarters with *C. bovis* infections were more resistant to infection by *Staph. aureus* than bacteriological-negative quarters (Pankey et al., 1985; Lam et al., 1997b).

The percentage of culture-negative samples of cases of both CM and SCM in The Netherlands has been determined to be approximately 25% (Barkema et al., 1998). One explanation for this high percentage might be a low concentration of udder pathogens, because the pathogen is either readily eliminated from the mammary gland (e.g. *E. coli*) (Erskine et al., 1991) or the pathogen invades into mammary epithelial (e.g. *Staph. aureus*)

(Dego et al., 2002). Another explanation might be the presence of viruses instead of bacteria. In spite of the fact that viruses are generally considered not to play an important role in the aetiology of bovine mastitis, Wellenberg et al. (2002) reviewed the updated evidence to demonstrate that viral infections are associated in a direct or indirect way with bovine mastitis. Bovine herpesvirus 1, bovine herpesvirus 4, foot-and-mouth disease virus and parainfluenza 3 have been isolated from milk cows with naturally occurring cases of CM. Viruses can namely induce teat lesions, which result in a reduction of the natural defence mechanisms of the udder, and indirectly contribute to bovine mastitis due to bacterial pathogens.

Pathogen-specific severity of inflammatory response. Not all bacteria cause an equally severe inflammatory response. *Escherichia coli* infections are often short-term infections, and are hardly ever subclinically present in the mammary gland (Lam 1996; Vaarst and Enevoldsen, 1997). Therefore, the innate immune system appears to play an initial key role. Cases of CM associated with culture-negative samples show generally strong similarities with clinical coliform mastitis (Vaarst and Enevoldsen, 1997). This is in sharp contrast to *Staph. aureus*, causing mostly chronic subclinical infections with relatively few clinical cases (Sears et al., 1990; Daley et al., 1991). Chronic infections occur when bacterial killing is not efficient. The adaptive immunity appears to be the major mechanism to cure these infections, although from some pathogens it is also known that spontaneous cure hardly ever occurs; e.g. *Staph. aureus*. *Staphylococcus aureus* invades into mammary epithelial and survives there (Dego et al., 2002). Eventually, the pathogens appear again in the lumen of the mammary gland, and another inflammatory response is mounted. Therefore, IMI with *Staph. aureus* can also be characterised by a wave-like pattern of increased SCC (Daley et al., 1991). For streptococci (e.g. *Strep. dysgalactiae* or *Strep. uberis*) not one particular pattern has been found, but they tend to be associated with longer increased SCC (Vaarst and Enevoldsen, 1997; Leigh et al., 1999). It may be of great biological value to interpret these SCC patterns in a lactation.

Factors affecting incidence of CM. The incidence of CM is influenced by several, non-management, effects, like season, parity, stage of lactation, breed, milking speed and udder conformation.

Season. Overall mastitis is detected with approximately equal frequencies throughout the year (Dohoo et al., 1983; Schukken et al., 1989), but there are pathogen-specific differences among seasons. For example, *E. coli* is most frequently isolated between May and July and between November and February (Schukken et al., 1989; Lafi et al., 1994). *Streptococcus*

uberis is found mainly in July and August (Schukken et al., 1989), while *Staph. aureus* is isolated most frequently in November and December (Schukken et al., 1989; Fox et al., 1995). Other studies have also shown a higher incidence of mastitis in winter months, compared with summer months (Pearson and Mackie, 1979; Schukken et al., 1989), and these seasonal variations suggest an influence of an increased number of micro-organisms in bedding material in the winter (Schukken et al., 1990).

Parity. Parity has been identified as a risk indicator for CM in several observational studies (Schutz et al., 1990; Weller et al., 1992; Laevens et al., 1997; Barkema et al., 1998; Pryce et al., 1999). All these studies reported that the incidence of CM increased with increasing parity.

Stage of lactation. Stage of lactation also influences the risk on occurrence of CM, which even differed among heifers and multiparous cows (Barkema et al., 1998). A higher rate of CM during the first month of lactation is observed in low SCC herds, but conversely, among the high SCC herds, an effect of stage of lactation is not observed (Erskine et al., 1988). Martin et al. (2002) concluded that there are pathogen-specific differences in the course of lactation. High SCC in early lactation is most likely caused by presence of minor pathogens, primarily CNS, whereas high SCC of cows being more than 90 days in milk (DIM) is primarily caused by *C. bovis*. The prevalence of major pathogens in quarters with ≥100,000 cells/ml decreased in the course of lactation.

Breed. Only slight differences between breeds are found. Some Scandinavian studies concluded that Friesian cows appear to be more prone to infection than the Swedish Red and White breed, the Ayrshires or the Finncattle (Batra, 1978; Schwan and Holmberg, 1979; Lindström et al., 1981; Ettala and Virtanen, 1990). On the other hand, Koenen et al. (1994) reported a decreasing incidence of CM for cows with a high proportion of imported Holstein genes in the Swedish Friesian breed. In a Dutch study a lower incidence of CM was found for Holstein-Friesian cows than in the Meuse-Rhine-Yssel breed (Schukken et al., 1990). This difference is even pathogen-specific as the Meuse-Rhine-Yssel breed is one of the risk factors for higher rates of clinical *Staph. aureus* mastitis, but not for clinical *E. coli* mastitis (Elbers et al., 1998). The Dutch average breeding values for SCC are, however, not different for the Holstein-Friesian, Dutch-Friesian and Meuse-Rhine-Yssel breed (NRS, 2002), but although CM and SCC are correlated traits, they are not identical traits.

Milking speed. Milking speed was found to be unfavourably correlated with lactation-average SCC but not with CM; i.e. fast milking cows had higher lactation-average SCC (genetic correlation = 0.44), but no genetic relationship was observed for CM (genetic correlation = 0.06) (Rupp and Boichard, 1999). However, Waage et al. (1998) showed that heifers with a low milk flow rate are more susceptible to CM than heifers with a medium to high flow rate.

Udder conformation. High favourable genetic correlations are estimated between teat length, udder depth, fore udder attachment and udder balance and cases of CM in several populations of dairy cows (Lund et al., 1994; Van Dorp et al., 1998; Rupp and Boichard, 1999; Sorensen et al., 2000). Cows with shorter teats, higher udders and tighter fore-udder attachment were genetically less likely to develop mastitis.

Somatic cells

Somatic cells are indicators of both resistance and susceptibility of cows to IMI. Milk somatic cells consist of several cell types, including polymorphonuclear leukocytes (PMN), macrophages, lymphocytes and a smaller percentage of epithelial cells. In healthy lactating mammary glands, epithelial cells are the predominant cell type, whereas PMNs are the major cell population during early inflammation. They (PMNs) play a protective role against infectious diseases in the bovine mammary gland (Miller et al., 1991; Kehrli and Shuster, 1994; Leitner et al., 2000; Riollet et al., 2000). The major roles of PMNs are to phagocyte and destroy infectious agents. Bacteria that pass the teat canal (= first line of defence) enter the teat cistern and meet the second line of defence; i.e. the phagocyte leukocytes (Detilleux et al., 1997). Once an inflammatory response has been initiated, PMNs are the first cells to be recruited to sites of infection. The recruitment of PMNs into infected mammary gland is a normal part of the cow's defence mechanism. It is very effective for eradicating the majority of infections that occur (Clarkson, 1975; Kehrli and Shuster, 1994; Riollet et al., 2000). In mammary glands that are infected with mastitis-causing pathogens, milk somatic cells consist for >95% of PMNs, and PMNs are thus indicators of inflammatory response (Detilleux et al., 1997; Pillai et al., 2001).

Typical lactation curve of SCC. A typical lactation curve for SCC starts off high shortly after parturition, decreases in the first 50 – 60 DIM to a lowest point, and increases slowly from then on towards the end of the lactation (Honkanen-Buzalski et al., 1981; Wiggans and Shook, 1987; Schutz et al., 1990; Weller et al., 1992). Sometimes, a curvilinear relationship was found, in which the SCC increased until approximately 250 DIM, and decreased slowly thereafter until the end of the lactation (Salsberg et al., 1984). Whereas Laevens et al. (1997) found a more or less equal SCC throughout the whole lactation, with a slight increase after 250 DIM.

The shape of the lactation curve for SCC differs among parities (Lederer and Kramer, 1980; Wiggans and Shook, 1987; Schutz et al., 1990; Weller et al., 1992; Schepers et al., 1997), and differs markedly between heifers and multiparous cows. For heifers, the lactation curve

is usually flat because of the smaller increase in SCC later in the lactation compared to the increase for multiparous cows. However, the significance of parity effect depends on what cows are included in the dataset. No effect was found when only bacteriological negative cows were considered, however, when all cows were included in the dataset, the effect of parity was significant (Laevens et al., 1997).

Effect of CM on typical lactation curve. Clinical mastitis can cause deviation from the typical lactation curve for SCC; i.e. one single high SCC, or a chronically high SCC. Lactation-average SCC ignores these differences, and cows may have the same lactation-average SCC, but with different patterns. The individual lactation curves might, therefore, provide more information to decrease the susceptibility of (some forms of) IMI. Based on the pathogenesis, each pathogen has its own typical characteristics, such as: (1) the time lag before SCC increases, and (2) the duration of increased SCC. These expected differences are confirmed by studies that either induced IMI, or analysed naturally occurring cases of CM. From experiments which induced IMI, it was shown that two days after inoculation with *E. coli*, SCC peaks, and the preinfection value is approached within three to four weeks again (Erskine et al., 1992; Pyörälä et al., 1994). However, within 24 h after the inoculation with *Staph. aureus*, SCC increases and remains high for at least 48 days (Shoshani et al., 2000).

Three other studies investigated the effect of infection status on cow SCC under practical circumstances (Sheldrake et al., 1983; Heuven, 1987; Schepers et al., 1997). Sheldrake et al. (1983) compared lactation curves for SCC of quarters free from CM with lactation curves for SCC of quarters with clinical *Staph. aureus*, CNS, and *C. bovis* mastitis. Particularly quarters with clinical *Staph. aureus* mastitis showed a considerable increase in SCC, but for all quarters with known infection status SCC was higher compared with quarters free from CM. Schepers et al. (1997) showed how different pathogens caused a different increase in quarter SCC. The largest increase was found for *Staph. aureus* and the smallest for *C. bovis*. Heuven (1987) showed that cows with only minor pathogens in their udder had just slightly higher levels of SCC than non-infected samples. Milk samples of cows with major pathogens showed much higher SCC levels when compared to either non-infected samples or samples with minor pathogens. Infections with major pathogens were more persistent, especially in older cows.

Reduction of incidence of clinical mastitis

Incidence of CM and prevalence of SCM on a farm or in a population can be reduced by changes in management, such as hygienic measures. These management changes are often combined in mastitis control programs (Park and Morgan, 1981; Oliver and Mitchell, 1984). A further option to reduce mastitis is genetic selection. Genetic selection might improve the genetic resistance of animals (Eriksson and Wretler, 1990) and therefore reduce mastitis in the long-term. Breeding for enhanced disease resistance is a relatively simple and readily available method to improve animal welfare and productivity in a variety of situations, as is reviewed by Stear et al. (2001). Even if unfavourable relationships exist, they do not present a fatal flaw in breeding schemes as breeders can create selection indices that contain traits with unfavourable associations (Cameron, 1997). In addition, breeders can select for resistance to several diseases simultaneously, perhaps by breeding for enhanced immune responsiveness (Wilkie and Mallard, 2000).

Mastitis control programs. The goal of the standard mastitis program is to reduce the number of new infections, and to limit the duration of the existing infections. The foundation for the basic "five-point mastitis control program" is laid in the 1960s (Neave et al., 1969). The program generally consisted of (1) optimisation of the milking procedures and optimal functioning of the milking machine, (2) application of teat disinfectant after removal of the milking unit, (3) dry cow treatment with antibiotics for all cows, (4) adequate treatment and documentation of all cases of CM, and (5) culling of chronically infected cows (Neave et al., 1969). These control measures reduced the prevalence of SCM (Grommers, 1981) by decreasing the incidence of new IMI and shortening the length of IMI (Natzke, 1981). The implementation of the five-point mastitis control program has led to control of *Strep. agalactiae* mastitis and, to a lesser extent, *Staph. aureus* and *Strep. dysgalactiae* mastitis (Neave et al., 1969; Hillerton et al., 1995). However, the program is less successful in preventing new infections with environmental pathogens (Schukken et al., 1990; Lam et al., 1997a). Recommendations to control both contagious and environmental pathogens have been combined in a ten-point mastitis control program, issued by the National Mastitis Council (2001). This program encompasses: (1) establishment of goals for udder health, (2) maintenance of a clean, comfortable environment, (3) proper milking procedures, (4) proper maintenance and use of milking equipment, (5) good record keeping, (6) appropriate management of CM during lactation, (7) effective dry cow management, (8) maintenance of biosecurity for contagious pathogens and marketing of chronically infected cows, (9) regular monitoring of udder health status, and (10) periodic review of mastitis control program. So, it includes segregation of infected and non-infected animals (Wilson et al., 1995), and recommendations with respect to biosecurity (Fenlon et al., 1995) in addition to measures mentioned in the five-point mastitis program.

Genetic selection. Genetic selection is merely on increased milk production, but the unfavourable correlations between milk yield and CM suggest that selection solely for yield will increase the CM incidence (Syvajarvi et al., 1986; Uribe et al., 1995). This effect can be counteracted by simultaneous selection on higher milk production and lower SCC. Genetic studies of records on veterinary treatments in the Nordic countries have shown that it is possible to improve the disease resistance by breeding (see review by Heringstad et al. (2000)). Selecting on increased genetic resistance of CM can be done directly or indirectly. Direct selection means that the actual trait is measured on the animal or its relatives. Indirect selection means that an indicator trait for CM is measured on the animal itself or its relatives. Direct selection to reduce the incidence of mastitis is the current practice in only the Nordic countries (Denmark, Finland, Norway, and Sweden). The major handicap for direct selection is the absence of disease recording in most other countries. Heritability estimates of CM are generally below 0.05 (Emanuelson, 1988; Weller et al., 1992; Mrode and Swanson, 1996), but considerable variation between bulls exists (Pryce et al., 1998). Lactation-average SCC is an important indirect measure for CM. It is the trait most commonly used, because it is readily available through most milk recording systems and it is related to mastitis. Moderately high to high genetic correlations have been estimated between CM and SCC (0.3 – 0.9), indicating that selecting purely on lower lactation-average SCC would increase the CM resistance (Emanuelson, 1988; Weller et al., 1992; Welper and Freeman, 1992; Zhang et al., 1994; Mrode and Swanson, 1996). Even pathogen-specific effects are found, as genetic selection on lower SCC reduces severity and duration of clinical episodes from especially environmental organisms during first lactation (Nash et al., 2002).

Next to SCC, conformation traits are widely used as indirect indicators of CM. Linear type traits represent descriptions of udder characteristics of a cow. They are scored nation-wide and therefore cheaply available for genetic selection. Genetic correlations between CM, SCC and type traits are generally low, but indicate a higher risk for CM with a weak udder suspensory, deep udders, bad front teat placement, loose rear udder attachment and long teats (Lund et al., 1994; Rogers et al., 1998; Van Dorp et al., 1998; Rupp and Boichard, 1999; Sorensen et al., 2000). In The Netherlands, milking speed is also included in the udder health index.

Molecular genetics. Traditional methods of selection have difficulties improving traits of low heritability, such as disease resistance. A great majority of disease resistance traits are likely to be influenced by the aggregate of many genes, each with a relatively small effect (Kelm et al., 2001). Recently, efforts have been undertaken to locate genes affecting economically important traits in dairy cattle. Genetic markers associated with these genes can be used in marker-assisted selection to increase genetic progress (Kashi et al., 1990). Application of marker-assisted selection for milk production and other economically

important traits would increase the rate of improvement for these traits. The use of genetic markers allows selection on the basis of a DNA profile. However, to find genetic markers for mastitis resistance, large datasets are required, which is a problem for most countries, except maybe the Nordic countries (Klungland et al., 2001).

Chromosome 23. Chromosome 23 contains the BoLA locus (Weigel et al., 1990; Dietz et al., 1997; Starkenburg et al., 1997; Ashwell et al., 1998). Dietz et al. (1997) identified an allele at the BoLA locus as a potential risk factor for acute IMI (= allele DRB3.2*16). This allele was also significantly associated with lower SCC in Holsteins and higher estimated breeding values for SCC (Kelm et al., 1997; Sharif et al., 1998). Several other alleles at the BoLA locus were reported to be associated with a decreased number of cases of CM, a smaller amount of discarded milk, and lower udder health costs (Weigel et al., 1990; Kelm et al., 1997; Sharif et al., 1998). These relationships suggest that alleles at the BoLA locus may serve as markers for health and production traits (Weigel et al., 1990). Sharif et al. (1999) concluded that increasing or decreasing the frequency of BoLA alleles to increase resistance to CM did not have adverse effects on production in the population they studied.

Indications for markers affecting milking speed and fore udder attachment were identified on chromosome 23 (Schrooten et al., 2000). These traits are genetically correlated to resistance of CM (Lund et al., 1994; Mrode et al., 1998; Sorensen et al., 2000).

Other chromosomes. It could be argued that an increased incidence of CM is caused by high milk yield, rather than a change in the immune response. Because of the unfavourable genetic correlation between milk production and incidence of CM, it can be expected that some markers affecting mastitis are positioned close to markers of milk production traits (Klungland et al., 2001). A number of markers affecting milk production traits have been published in different breeds, of which several map to chromosome 6 (Ashwell et al., 1996; Spelman et al., 1996; Schrooten et al., 2000). Chromosome 7 may contain a marker which affects SCC (Heyen et al., 1999; Ashwell et al., 2001), but markers affecting SCC were also identified on chromosome 5, 21, 22, 23 and 26 by Heyen et al. (1999).

Shortcomings of current use of somatic cell count

Management. So far, the aim of most mastitis control programs is to lower lactation-average SCC in order to monitor udder health. Herd-specific mastitis control programs are based on the continuous monitoring of primary parameters, of which the bulk milk SCC and the proportion of SCC test-day recordings >250,000 cells/ml are currently the most important ones in The Netherlands. Both primary parameters are presented on the current forms of the national milk recording system (NRS, Arnhem, The Netherlands), and special

attention is put on cows with >250,000 somatic cells/ml, and heifers with >150,000 somatic cells/ml. Because of the differences in severity of the several mastitis-causing pathogens, it can be expected that especially the short-term infections are not detected by these two parameters. Therefore, it can be questioned if it is possible to direct the management advises specifically on lowering the incidence of pathogen-specific CM, or shortening the duration of infection based on the currently used primary parameters.

Genetics. Kehrli and Shuster (1994) argued that cows with very low SCC might be more susceptible to CM, because their ability to respond to IMI would be reduced. However, other results indicated that cows with the lowest initial SCC had the lowest risk for first CM, without any intermediate optimum (Rupp and Boichard, 2000). The linear relationship between sires' breeding values for CM and for SCC indicated that the lower lactation-average SCC, the lower the incidence of CM throughout the full range (Philipsson et al., 1995). Therefore, SCC should be decreased to the lowest possible value, at least within the range covered by the genetic variance. This agrees with conclusions of Rupp et al. (2000), who concluded that cows with the lowest mean SCC in the first lactation had the lowest risk for CM in the second lactation. It suggests that genetic selection for decreased SCC may effectively reduce incidence of CM and that breeding goals should favour cows with the lowest observed SCC. However, although it is known that aetiology of pathogens differ considerably, little is known about the genetic variation of incidences of the mastitis-causing pathogens. Therefore, it can be questioned if the current selection indices, that realise an increase in milk yield and simultaneously monitor udder health by selecting for lower lactation-average SCC, improve resistance to only some or to all pathogens.

Alternative use of somatic cell count

Average lactation values of SCC are generally used in mastitis control programs and for genetic improvement of udder health. However, Detilleux et al. (1997) concluded that analyses of SCC as candidate for selection against mastitis resistance could be improved by choosing better measures of SCC. These measures should contain non-genetic factors that cause variation in SCC and methods of genetic epidemiology could be used as well. Depending upon the goal of the study, various ways of using SCC may be proposed for udder health surveillance. Examples proposed by Detilleux et al. (1997) were (1) proportion of test-day SCC above or below a certain limit, (2) direction and rate of change in test-day SCC, (3) time until SCC reach a given limit, (4) difference between observed SCC and SCC expected under healthy conditions, (5) area under (parts of) the lactation curve of SCC, (6) rolling averages, and (7) DIM that the increase in SCC happens. When associating these

SCC traits to the occurrence of CM it should be taken into account that recording an increased SCC on test-days depends on (a) the day of occurrence of CM in relation to the test-day recordings and (b) the duration of increased SCC as a result of a case of CM. Both the day of occurrence and the duration of increased SCC might be pathogen-specific.

Suggestions for other traits have been given in other studies as well (Heuven, 1987; Schepers et al., 1997). Schepers et al. (1997) also provided alternative measures of SCC, based on the evaluation of the thresholds for IMI based on SCC. Twelve alternative SCC test statistics were calculated, divided in three groups: (1) identification of IMI was based on three different fixed SCC values, (2) five thresholds, that were specific to parity, for which identification of IMI was based on the lactation curve of SCC, (3) four thresholds for which identification of new IMI was based on deviation between current and previous samples in the same lactation. The use of SCC thresholds for specific parities and stages of lactation to detect IMI improved the quality of parameters only slightly over a fixed threshold of 200,000 cells/ml (Dohoo and Leslie, 1991), which is generally used as the optimum threshold for predicting new infections.

Finally, Heuven (1987) analysed test-day records of SCC to predict the presence of mastitis-causing pathogens, and developed a method to identify abnormal observations of SCC, in order to exclude them from the dataset. An observation was considered to be abnormal on the basis of its deviation from the normal lactation curve. While using this exclusion method, it was concluded that cows with either a high average SCC or a test-day with a high deviation from the typical lactation curve for SCC were more likely to be treated for CM. A single test-day with a high SCC recorded may not affect lactation-average SCC much, whereas longer increased SCC will affect lactation-average SCC eventually. Therefore, by selecting for lower lactation-average SCC, the group of cows with a single high deviation from the typical lactation curve for SCC might be missed. However, these cows might still be more genetically susceptible to CM, as they might become infected more often, making it more likely that they would have elevated SCC on a single test-day.

Lactation-average SCC ignores dynamics of PMN emigration in response to infection. Some cows react rapidly to infection and cure the infection because high numbers of phagocytically active blood PMN migrate rapidly in milk. Others do not respond as rapidly to IMI and have moderate to high SCC for long periods (Riollet et al., 2000; Paape et al., 2002; Van Oostveldt et al., 2002). Nevertheless, both types of cows can have similar lactation-average SCC. Variation during lactation among test-day records for SCC is currently most often not taken into account. However, it is expected that the longitudinal SCC data provide additional information about the pathogens involved in cases of CM (Reents et al., 1995a; Reents et al., 1995b).

Chapter 1

Outline of this dissertation

The ultimate objective of this thesis is to develop a method that uses variation and patterns in test-day records of SCC to identify pathogen-specific CM incidence in animals that are grouped either per herd or per sire. This objective can be split up in five parts:

- Current selection indices realise an increase in milk yield and simultaneously monitor udder health by selecting for lower lactation-average SCC. To analyse whether these indices affect all pathogen-specific cases of CM equally or not, the genetic variation for overall and pathogen-specific CM is quantified in Chapter 2, and genetic correlations with milk production and SCC are estimated.

- So far, lactation-average SCC is generally used in mastitis control programs and for genetic improvement of udder health, but these average lactation values of SCC ignore variation in SCC during lactation. Therefore, pathogen-specific effects on SCC during lactation are described in Chapter 3, resulting in patterns of SCC before and after cases of pathogen-specific CM.

- Additional information about the pathogens involved in cases of CM can be provided by using longitudinal SCC data. Test-day records of SCC can be used more effectively by defining patterns of peaks in SCC during the lactation. These SCC patterns are defined in Chapter 4, and their phenotypic associations with occurrence of pathogen-specific cases of CM are estimated in this chapter.

- Genetic parameters for pathogen-specific CM and the patterns of peaks in SCC are estimated in Chapter 5. This to establish if these SCC patterns do provide additional information for genetic selection that aims to decrease genetic susceptibility to pathogen-specific CM, in comparison to the information provided by lactation-average SCC alone.

- Associations between incidence rates of pathogen-specific CM and of patterns of peaks in SCC on herd level are determined in Chapter 6. This to investigate if health management advises can be directed specifically on lowering the incidence rate of pathogen-specific CM, or shortening the duration of infection, based on the information provided by the incidence rates of the SCC patterns.

The general discussion, Chapter 7, deals with the future prospects of the improvement of udder health by applying the patterns of peaks in SCC in genetic selection or in mastitis control programs.

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Chapter 2

Genetic parameters of pathogen-specific incidence of clinical mastitis in dairy cows

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Abstract

Data from 274 Dutch herds recording clinical mastitis (CM) over an 18-months period were used to quantify the genetic variation for overall and pathogen-specific CM. Analysed pathogens were *Staphylococcus aureus*, coagulase-negative staphylococci (CNS), *Escherichia coli*, *Streptococcus dysgalactiae*, *Streptococcus uberis* and other streptococci. The dataset contained 47,563 lactations of 28,695 cows of different parities. Cases of overall and pathogen-specific CM were treated as all-or-none traits. Variance components for the sire, maternal grandsire, and permanent environmental effect were estimated using generalised linear mixed models with a logit link function for the binary traits. Average number of days at risk and in milk on trial was 198 days. The estimated heritability for overall CM was 0.04, and similar heritabilities for the pathogen-specific CM were estimated. Repeatability across lactations was low for overall and pathogen-specific CM (0.10 to 0.14). Genetic correlations with milk yield and somatic cell score (SCS) differed according to pathogen. For instance, the incidence rate of clinical *E. coli* mastitis was slightly unfavourably correlated with milk yield at 150 days (0.13) but stronger with SCS (0.74). Whereas, the genetic correlations with clinical *Strep. dysgalactiae* mastitis were 0.70 and 0.16, respectively. The expected correlated responses showed that current selection practices (using milk yield and SCS) will be effective in reducing the incidence of *E. coli* and CNS but less effective in reducing the incidence of *Staph. aureus* and *Strep. dysgalactiae*, even with a large relative weight for SCS in the selection index.

Keywords Dairy cattle, Clinical mastitis, Pathogens, Heritabilities, Genetic correlations

Introduction

Clinical mastitis (CM) is one of the major diseases in dairy herds. It induces economic costs, mainly consisting of discarded milk, increased health care costs and reduced milk quality (Timms and Schultz, 1984; Heuven, 1987). Mastitis also contributes to consumer concerns regarding animal welfare (Willeberg, 1994) and concerns regarding the impact of use of antibiotics in animals on efficacy of antibiotics for human health. Mastitis control programs are designed to reduce (sub)clinical mastitis on farms, as these provide guidelines for hygiene and management practices to control intramammary infections with contagious pathogens (Neave et al., 1969). Genetic selection is another strategy to combat (sub)clinical mastitis. Although genetic selection is a slow process, it results in a permanent change in the genetic composition of the dairy herd (Shook, 1989).

Heritability estimates of CM are generally below 0.05 (Emanuelson et al., 1988; Weller et al., 1992; Mrode and Swanson, 1996; Pryce et al., 1998), but considerable variation between bulls exists (Pryce et al., 1998). The genetic correlation between milk yield and CM is unfavourable (Syvajarvi et al., 1986; Groen et al., 1994; Uribe et al., 1995), suggesting that selection solely for yield will increase the CM incidence. Selection on breeding values for CM, instead, might counteract this unfavourable correlated response (Pryce et al., 1998). In the absence of direct records of CM, indirect measures of (sub)clinical mastitis are often used to assess genetic variation for susceptibility to mastitis. Somatic cell count (SCC) or log transformed SCC (Ali and Shook, 1980), is the primary trait used for indirect selection for genetic resistance to both clinical and subclinical mastitis. Heritability estimates of log transformed SCC are higher than for CM, with a mean of 0.10 and 0.04, respectively (Emanuelson et al., 1988; Weller et al., 1992; Mrode and Swanson, 1996). The estimated genetic correlations between CM and SCC are moderately high to high (0.3 – 0.9), indicating that selecting for lower SCC would increase the resistance to CM (Mrode and Swanson, 1996; Emanuelson, 1997). Other traits that are used as indicators of CM are udder conformation and milking speed (Lund et al., 1994; Boettcher et al., 1998; Rupp and Boichard, 1999).

Several pathogens play a crucial role in the development of CM. The most frequently isolated pathogens from clinical cases in The Netherlands are *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus dysgalactiae* and *Streptococcus uberis* (Schukken et al., 1989; Miltenburg et al., 1996; Barkema et al., 1998). Differences in incidence of pathogen-specific CM at different levels of SCC have been shown but, so far, the association between pathogen-specific CM and SCC has only been evaluated on the level of bulk milk SCC (BMSCC) (Erskine et al., 1988; Miltenburg et al., 1996; Barkema et al., 1998). However, little is known about the genetic variation of incidences of these pathogens and whether selection for yields and SCC affects all pathogens equally or not. And, therefore, does a lower SCC improve resistance to only some or to all pathogens, since the aetiology of each

mastitis-causing pathogen is different (Rogers et al., 1995; Nash et al., 2000). This ignorance is primarily due to lack of data. Therefore, the objectives of this study were to quantify genetic variation for overall and pathogen-specific CM and to estimate genetic correlations with milk production and SCS.

Material and Methods

Available data. Records on CM were available from an experiment carried out from December 1992 till June 1994 on 274 Dutch farms (Barkema et al., 1998). The actual start and end date of the study varied slightly among farms but all farms participated in the study for 18 months. Lactating cows were housed in free-stall barns and milking parlours were double herringbone or two-sided open tandem shape. Herds participated in the milk recording system, and annual milk production quota was between 300,000 and 900,000 kg. The national milk recording system (NRS, Arnhem, The Netherlands) provided information of milk recordings of all cows participating in the study, recorded every third of fourth week. A record included national cow identification, breed, date of milk recording, date of calving, date of drying off, test-day milk yields (milk, fat and protein (all in kg)) and test-day somatic cell count. The breed of the cow was subdivided into three main contributing breeds, with each having up to nine classes (0, 1/8, …, 8/8) depending on the degree of contribution. The scores of these three breeds always summed up to 8/8, and the main breeds were Holstein-Friesian (HF), Dutch-Friesian (FH) and Meuse-Rhine-Yssel (MRY).

While participating in the study, farmers were asked to collect milk samples from every quarter that they saw with visible signs of CM. The samples were stored in a freezer on the farm (at approximately -20°C) and were collected for bacteriological examination at intervals of six to eight weeks. Collected data contained information on the national cow identification, buying and selling date of the cow, date of occurrence, infected quarter, and the outcome of the bacteriological culturing of the milk samples.

A pedigree file of all cows on the participating herds was available and contained the ancestry of approximately 240,000 cows and 15,000 bulls back to 1937.

Data editing. A dataset with information on CM and bacteriological characteristics was constructed from the phenotypic records. While constructing the dataset, the aim was to include the maximum feasible number of lactations recorded during the study and to avoid bias due to culling for CM in early lactation. Therefore, every lactation that was recorded for at least one day during the experiment was included in the dataset, which were in total

49,529 lactations (Table 2.1). The number of days at risk and in milk for each lactation was determined, requiring a start and end date. The start date was that occurring latest: (1) calving date, (2) starting date of the study on the farm or (3) buying date of the cow at that farm. The end date was that occurring first: (1) drying off date, (2) date of 450 days post partum, (3) end date of the study on the farm or (4) selling date of the cow at that farm. Subtraction of the end date from the start date resulted in the number of days at risk and in milk during the trial for each lactation (days on trail; DOT). Number of days in milk at the start of the study (days at start; DAS) was also determined by subtracting the calving date from the starting date of the study on the farm. A zero was scored when the cow was not yet in milk at the start of the study. These two variables were in fact constructed to be able to adjust for the variable length of DOT and DAS between cows due to a fixed sampling period of health data. Approximately 35% of the lactations were already started before the experiment and the average DAS was 58 days, with an average DOT of 198 days.

Analysed udder health traits were CM and the results of the bacteriological culturing. The udder health traits were categorical, so when in a lactation at least one case of CM was recorded, it was registered as 1, otherwise it was scored as 0. The same strategy was used for grouping of pathogens (1 = presence, 0 = absence), irrespective of whether the cow had one or more than one case of CM during the lactation. Therefore, a cow could be scored with a 1 for more than one pathogen in one lactation. Also, no difference was made between separate cases of CM with different pathogens and one case with a mixed culture. More than one case of CM occurred in 1,442 out of 5,950 lactations with CM. Only the most frequently isolated pathogens were analysed, and the streptococci other than *Streptococcus dysgalactiae* and *Streptococcus uberis* were grouped together to increase frequency. Pathogens studied were S*taphylococcus aureus*, coagulase-negative staphylococci (CNS), *Escherichia coli*, *Streptococcus dysgalactiae*, *Streptococcus uberis* and other streptococci. In 14.7% of all bacteriological examinations no pathogen could be isolated (culture-negative).

Production traits were averaged from test-day records of kg milk (MILK), kg fat (FAT), kg protein (PROT) and somatic cell score (SCS) ($SCS = \log_2(SCC/100{,}000)+3$). Each production trait was averaged over the test-day records up to 150 and 305 days in lactation, respectively. An average over the first 150 days was calculated if a cow had three or more recordings of SCS, and four or more of MILK, otherwise a missing value was assigned. Similarly, missing values were replaced with averages over the first 305 days when SCS was measured at least six times and MILK at least seven times. Projected 305-day yields for milk, fat and protein were available from the national milk recording system and were analysed as production traits as well.

For the analyses, a pedigree file was constructed based on sires and maternal grandsires of cows in the data. This file contained 3,285 AI bulls with 2,073 sires plus 1,934 maternal

grandsires (of which 1,068 were sire as well), and 346 unique identities of fathers of the sires or maternal grandsires. The identification of the bull's mother was only included when the cow had two or more sons in the pedigree file, otherwise she was included as a base parent. Cows with unknown pedigree were deleted from the dataset, reducing the dataset to 47,988 lactations (Table 2.1).

Final editing was done by excluding cows with extreme ages at calving (for a given parity). Boundaries were established using histograms; first parity cows had to calve between 490 and 1,250 days, second parity cows between 790 and 1,525 days, third parity cows between 1,100 and 1,830 days, and fourth or later parity cows after 1,400 days. This reduced the dataset to 47,563 lactations from 28,695 cows (Table 2.1).

Table 2.1 *Effect of data editing of the health dataset on the number of lost lactations (lost) and the total number of lactations, herds, cows and all-or-none clinical mastitis cases (CM)*

	lost	lactations	herds	cows	CM
Only lactations with ≥1 day on trial		49,529	274	29,882	6,175
Only cows with known pedigree	1,541	47,988	274	28,825	6,012
Only cows with correct ages at calving	425	47,563	274	28,695	5,950

Incidence rate of clinical mastitis. Incidence rates of clinical mastitis (IRCM) were expressed per cow-day at risk. Cow-days at risk were calculated as the total number of days that the cows were at risk and in milk during the experiment (= sum of DOT over all lactations = 9,404,452 days). Calculations of overall CM included the total number of lactations with at least one case of CM. For pathogen-specific CM it included the number of lactations with at least one pathogen-specific case. Dividing this by the sum of DOT resulted in overall or pathogen-specific IRCM (Rothman and Greenland, 1998).

Statistical analyses. AS-REML (Gilmour et al., 2000) was used to estimate variance components, using generalised linear mixed models with a logit link function. Univariate analyses were carried out for production traits (MILK, FAT, PROT and SCS) using a linear model (Y), and for udder health traits (overall plus pathogen-specific CM) using a logistic model (Logit(Y)). Cows with missing values for MILK, FAT, PROT or SCS were still included in the analyses, since these cows were culled early in the lactation. The model included

random effects for sire and maternal grandsire (MGS) and for cow, to account for permanent environment across repeated lactations. The model used was:

$$Y \text{ or } Logit(Y) = \mu + \text{fixed effects} + S_{sire} + \tfrac{1}{2} S_{mgs} + PERM_{animal} + e$$

The random sire effect was identified by the subscripts for sire and MGS; S_{sire} and S_{mgs}, respectively. The sire effects were linked using the relationship matrix, and were assumed to be normally distributed with $var(S_{sire\ or\ mgs}) = \sigma^2_s$. The permanent environmental effect was assumed to be normally distributed, with $var(PERM_{animal}) = \sigma^2_{Ep}$. For the logistic model, the residual variance (σ^2_e) was fixed on 3.29; N(0, 3.29) (Gilmour et al., 2000).

Fixed effects included were herd (with 274 levels), an interaction between year and season of calving (YS, with 18 classes), parity (with four classes, where the last class contains all parities ≥4), HF and FH percentage (both with nine classes, for 0, 1/8, …, 8/8) and MRY percentage (with five classes, for 0, 1/8, 2/8, 3/8, ≥4/8). In the logistic model, polynomials were included for age at calving, DOT and DAS. In the linear model, only a polynomial for age at calving was included. Order of the polynomials was established by stepwise inclusion of higher order regression coefficients (forward and backward elimination), till the estimated regression coefficient did not differ significantly from zero any more (Table 2.2). For estimating the variance components for the udder health traits, it was decided to apply the order of fit of CM so all udder health traits were analysed with the same model. For the production traits a polynomial of order four for age at calving was established.

Table 2.2 *Highest significant levels of the estimated polynomials for age at calving (AGEC), days in milk at the start of the study (DAS) and the number of days on trial (DOT) from univariate analyses of pathogen-specific clinical mastitis traits*

	AGEC	DAS	DOT
Clinical mastitis	1	2	4
Staphylococcus aureus	1	1	2
Coagulase-negative staphylococci	1	1	1
Escherichia coli	1	1	1
Streptococcus dysgalactiae	1	1	1
Streptococcus uberis	1	1	2
Other streptococci	1	1	2
Culture-negative samples	0	1	2

Bivariate analyses were carried out to estimate correlations between udder health and production traits, using a combined logistic and linear model. Fixed effects for the logistic and linear model were the same as mentioned for the univariate analyses. (Co)variance matrices (2x2) were estimated for the sire, permanent environment and residual effects, but σ^2_{e1} was fixed on 3.29; N(0, 3.29) (Gilmour et al., 2000):

$$\begin{bmatrix} \sigma^2_{s_1} & \\ \sigma_{s_1\ s_2} & \sigma^2_{s_2} \end{bmatrix} \begin{bmatrix} \sigma^2_{Ep_1} & \\ \sigma_{Ep_1\ Ep_2} & \sigma^2_{Ep_2} \end{bmatrix} \begin{bmatrix} \sigma^2_{e_1} & \\ \sigma_{e_1\ e_2} & \sigma^2_{e_2} \end{bmatrix}$$

Calculation of genetic parameters. Genetic parameters were calculated from the estimated variance components. The additive genetic variance was calculated by multiplying the sire variance by four. The phenotypic variance (σ^2_p) was the sum of the sire variance multiplied by 1.25, where 1.25 was included because MGS was fitted in the model separately, plus the permanent environmental and residual variances. Division of the additive genetic variance by the phenotypic variance resulted in the heritability. The permanent environmental effect between lactations was calculated by dividing σ^2_{Ep} by σ^2_p. Genetic, phenotypic, permanent environmental and error correlations were estimated using the corresponding variances and covariances. For CNS it was difficult to estimate the covariance between the two permanent environmental effects ($\sigma_{Ep1\ Ep2}$), leading to convergence problems. Therefore, the model was simplified by fixing this component to zero when analysing CNS.

Results

The mean production of MILK, FAT, PROT and SCS in 150 and 305 days of lactation is shown in Table 2.3. Means of the projected 305-day yields for milk, fat and protein were similar to the mean of the averaged productions over the test-day records up to 305 days, and these were therefore not included in the table.

The overall IRCM was 0.00063 all-or-none cases per cow-day at risk, whereas the pathogen-specific IRCM ranged from 0.00005 to 0.00017 all-or-none cases per cow-day at risk (Table 2.4). Proportions of diseased lactations with increasing number of days at risk and in milk in the lactation (DOT) are shown in Figure 2.1 for (a) the original data, (b) the fitted values from the model, and (c) the fitted polynomial for DOT with the assumption DAS = 0 and mean values for all fixed effects. By setting DAS = 0, it is assumed that all cows entered the study directly after calving and not at different lactation stages. The difference between

the fitted polynomial curve for DOT and the histograms in Figure 2.1 was mainly due to fixing DAS at 0. Closer agreement with the estimated curve was obtained for a subset of the data, based on only those lactations that actually started within the experiment, i.e. DAS = 0.

Table 2.3 *Number of non-missing records for production traits (MILK, FAT, PROT, SCS) averaged over test-day records up to 150 or 305 days of lactation, phenotypic mean, minimum and maximum value, genetic (σ_a), permanent environment (σ_c) and residual (σ_e) standard deviations, heritability (h^2) and permanent environmental effect (c^2), all from univariate analyses, with their respective standard errors as subscripts*

	No.	Mean	Min	Max	σ_a	σ_c	σ_e	h^2	c^2
150 days									
MILK	42,816	28.80	11.06	58.36	2.14	2.42	2.71	$0.31_{0.03}$	$0.40_{0.01}$
FAT	42,816	1.26	0.38	2.63	0.07	0.10	0.13	$0.20_{0.02}$	$0.36_{0.01}$
PROT	42,816	0.96	0.33	1.71	0.05	0.07	0.08	$0.23_{0.02}$	$0.39_{0.01}$
SCS	36,996	2.42	-1.48	8.61	0.35	0.80	1.09	$0.07_{0.01}$	$0.34_{0.01}$
305 days									
MILK	41,149	24.95	9.00	49.85	2.06	2.31	2.14	$0.38_{0.03}$	$0.47_{0.01}$
FAT	41,149	1.11	0.41	2.08	0.07	0.09	0.10	$0.27_{0.03}$	$0.45_{0.01}$
PROT	41,149	0.86	0.35	1.54	0.05	0.07	0.07	$0.28_{0.03}$	$0.48_{0.01}$
SCS	36,814	2.77	-0.81	8.00	0.37	0.76	0.88	$0.10_{0.02}$	$0.41_{0.01}$

The heritabilities and permanent environmental effects from univariate analyses are shown in Table 2.3 for MILK, FAT, PROT and SCS and in Table 2.4 for (pathogen-specific) CM. The heritabilities for 150-day MILK, FAT and PROT were lower than the estimates for 305 days. The heritability for overall CM was 0.04, and the heritabilities for pathogen-specific CM ranged from 0.02 for other streptococci to 0.10 for CNS. Permanent environmental effects (c^2) were more important for production traits (ranging from 0.34 to 0.48) than for udder health traits (ranging from 0.04 to 0.10).

Phenotypic correlations between the udder health traits and MILK, FAT, PROT and SCS ranged from 0.01 to 0.31. The positive phenotypic correlations between udder health traits and MILK, FAT and PROT indicated that high-producing cows tended to have more cases of (pathogen-specific) CM. Comparison of the mean production yields of cows with and without CM confirmed this (30.1 *vs.* 28.6 kg milk, 1.31 *vs.* 1.25 kg fat, 1.00 *vs.* 0.95 kg

protein). The positive phenotypic correlations between SCS and the udder health traits indicated that cows with CM had higher SCC than cows without CM, which also was confirmed by the calculated means of SCS for both groups (3.27 *vs.* 2.31).

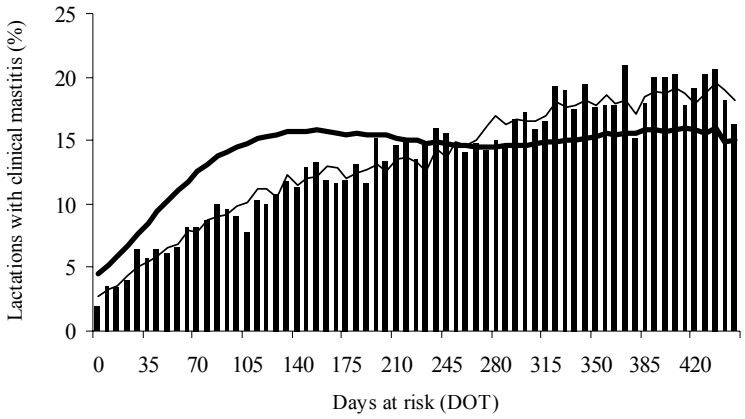

Figure 2.1 *Proportion of lactations with clinical mastitis for varying the number of days at risk and in milk per lactation (DOT) shown as histograms. The fitted values from the model are shown by the thin line and the fitted polynomial for DOT (assuming DAS = 0 and mean values for all fixed effects) is shown by the thick line.*

Permanent environmental correlations were hard to estimate and in 20 out of 64 runs they were fixed at the boundary, 1 or -1. The effect of this on the estimates of the other (co)variance matrices was small. When no boundaries were set, unrealistic permanent environmental correlations (>1) were estimated, but the genetic correlation did not change much. The other alternative was to fix the permanent environmental correlation to zero, which increased the genetic correlations only slightly, and reduced the phenotypic correlations.

Genetic correlations between CM and MILK (Table 2.5) were unfavourable, 0.69 for 150-day milk yield and 0.73 for 305-day milk yield. The genetic correlation between CM and 150-day SCS was 0.63 and it was 0.39 between CM and 305-day SCS. Genetic correlations between pathogen-specific CM and production traits were all unfavourable (Table 2.5).

Genetic correlations between pathogen-specific CM and MILK, FAT and PROT were higher for 305-day records than for 150-day records, with the exception for clinical *Strep. dysgalactiae* mastitis with all three production traits, and clinical *Strep. uberis* mastitis with MILK and PROT. The estimated genetic correlations of pathogen-specific CM with 150-day SCS were always higher than with 305-day SCS.

Table 2.4 *Number of cases for health traits (overall and pathogen-specific clinical mastitis), incidence rates of clinical mastitis (IRCM) per cow-day at risk, genetic (σ_a) and permanent environment (σ_c) standard deviations, heritability (h^2) and permanent environmental effect (c^2), all from univariate analyses, with their respective standard errors as subscripts*

	No.	IRCM	σ_a	σ_c	h^2	c^2
Clinical mastitis	5,950	0.00063	0.39	0.52	$0.04_{0.01}$	$0.07_{0.01}$
Staphylococcus aureus	1,597	0.00017	0.41	0.57	$0.05_{0.02}$	$0.09_{0.03}$
Coagulase-negative staphylococci	504	0.00005	0.58	0.38	$0.10_{0.06}$	$0.04_{0.08}$
Escherichia coli	1,485	0.00016	0.45	0.51	$0.06_{0.03}$	$0.07_{0.03}$
Streptococcus dysgalactiae	963	0.00010	0.44	0.45	$0.05_{0.03}$	$0.06_{0.04}$
Streptococcus uberis	517	0.00005	0.39	0.59	$0.04_{0.04}$	$0.10_{0.06}$
Other streptococci	714	0.00008	0.25	0.54	$0.02_{0.03}$	$0.08_{0.04}$
Culture-negative samples	1,121	0.00012	0.44	0.40	$0.06_{0.03}$	$0.05_{0.04}$

Discussion

In this study, presence or absence (1/0) of CM during parts of the lactation that coincided with the trial period was analysed. The same data was used by Barkema et al. (1998) for analysing the total number of clinical quarter cases per 365 days at risk, and they have calculated an IRCM of 0.26 per 365 days at risk. The reported IRCM per cow-day at risk is lower in the current study as more than one case of CM occurred in 24% of all lactations with CM. Barkema et al. (1998) concluded that IRCM increased as parity increased, from 4.38 to 13.88 quarter cases per 10,000 cow-days at risk for heifers and cows that had calved eight times, respectively. And they also showed the distribution of IRCM per week after calving for heifers and older cows, separately, which is high shortly after calving and decreases to a lower level within five weeks.

Table 2.5 *Estimated genetic correlations from bivariate analyses between overall and pathogen-specific all-or-none clinical mastitis recorded in the first 450 days of lactation, and production traits (MILK, FAT, PROT, somatic cell score (SCS)) averaged over test-day records up to 150 or 305 days of lactation, with their respective standard errors as subscripts*

	150 days				305 days			
	MILK	FAT	PROT	SCS	MILK	FAT	PROT	SCS
Clinical mastitis	0.69 $_{0.09}$	0.52 $_{0.11}$	0.54 $_{0.10}$	0.63 $_{0.05}$	0.73 $_{0.08}$	0.57 $_{0.10}$	0.62 $_{0.09}$	0.39 $_{0.15}$
Staphylococcus aureus	0.68 $_{0.14}$	0.40 $_{0.17}$	0.63 $_{0.14}$	0.45 $_{0.23}$	0.70 $_{0.14}$	0.46 $_{0.16}$	0.65 $_{0.13}$	0.27 $_{0.23}$
Coagulase neg. staphylococci	0.25 $_{0.22}$	0.34 $_{0.23}$	0.24 $_{0.21}$	0.99 *	0.26 $_{0.22}$	0.41 $_{0.21}$	0.29 $_{0.20}$	0.54 $_{0.36}$
Escherichia coli	0.13 $_{0.21}$	0.51 $_{0.19}$	0.07 $_{0.21}$	0.74 $_{0.18}$	0.25 $_{0.19}$	0.55 $_{0.16}$	0.24 $_{0.19}$	0.63 $_{0.17}$
Streptococcus dysgalactiae	0.70 $_{0.15}$	0.34 $_{0.20}$	0.42 $_{0.18}$	0.16 $_{0.27}$	0.63 $_{0.25}$	0.17 $_{0..22}$	0.33 $_{0.20}$	0.04 $_{0.27}$
Streptococcus uberis	0.44 $_{0.29}$	0.12 $_{0.33}$	0.21 $_{0.30}$	0.62 $_{0.48}$	0.41 $_{0.30}$	0.18 $_{0..31}$	0.15 $_{0.33}$	0.47 $_{0.46}$
Other streptococci	0.74 $_{0.30}$	0.44 $_{0.34}$	0.36 $_{0.28}$	0.99 *	0.79 $_{0.25}$	0.55 $_{0..31}$	0.57 $_{0.26}$	0.99 *
Culture-negative samples	0.41 $_{0.17}$	0.44 $_{0.19}$	-0.05 $_{0.23}$	0.34 $_{0.23}$	0.52 $_{0.15}$	0.50 $_{0.17}$	0.16 $_{0.20}$	0.26 $_{0.23}$

* These genetic correlations were put on the boundary by AS-REML.

Heritabilities. The heritabilities of the udder health traits in this study were estimated with a threshold model. In most studies, the estimates of heritabilities originate from analyses with a linear model (Emanuelson et al., 1988; Koenen et al., 1994; Pösö and Mäntysaari, 1996; Pryce et al., 1997). In other studies a generalised linear model has been applied to the underlying liability scale (Weller et al., 1992; Uribe et al., 1995; Heringstad et al., 1997). Robertson and Lerner (1949) showed that estimates from a linear model are frequency dependent and should be transformed from the observable to the underlying scale for comparison purposes.

The estimated heritability for overall CM using a threshold model (0.04) was in the low region of estimates in other studies using a threshold model (Simianer et al., 1991; Weller et al., 1992; Uribe et al., 1995; Heringstad et al., 1997). Heritabilities for pathogen-specific CM were similar to the CM-estimate, ranging from 0.02 (other streptococci) to 0.10 (CNS). Heritabilities for CM caused by environmental pathogens (*E. coli*, *Strep. dysgalactiae*, *Strep. uberis* and other streptococci) obtained an average of 0.04. This is contrasting Nash et al. (2000), who estimated higher heritabilities for CM incidences from the environmental organism groups (coliforms plus streptococci other than *Strep. agalactiae*), ranging 0.11 to 0.25 for heifers and 0.12 to 0.19 for second lactation cows.

Due to the binary nature of the pathogen data and the low incidences, observations of some levels of the fixed effects in the model can be all present or absent (1 or 0). This could create the "extreme category problem" that might bias the estimates of variance components, which results in suspiciously high heritabilities. Three techniques for dealing with this were investigated by Rekaya et al. (2000). One of their options was to group low incidence observations. In our study, grouping of the low incidence observations had no effect on the estimate when analysing clinical CNS mastitis, a trait with a low incidence. A heritability of clinical CNS mastitis of 0.10 was estimated, irrespective of whether the low incidence observations were grouped or not. The results of Rekaya et al. (2000) suggested however that grouping was not the most effective technique to reduce bias. Therefore, the high heritability for clinical CNS mastitis might still be biased by the low incidence rate.

Genetic correlations. In our study, genetic correlations between udder health traits and MILK, FAT, PROT and SCS were all antagonistic, but standard errors were large. Correlations of 0.63 and 0.39 were estimated between CM and SCS in the first 150 and 305 days, respectively. This suggests that selection for lower SCS, especially during early lactation, also decreases the incidence of CM. With selecting for MILK only, the largest effect may be for pathogen-specific CM caused by *Staph. aureus, Strep. dysgalactiae* or other streptococci, while clinical *E. coli* mastitis seems to be less affected. With genetic selection purely on decreased SCS, the number of cases of clinical *E. coli* mastitis will decrease most,

while the number of cases of clinical *Strep. dysgalactiae* mastitis may not be affected at all. Simultaneous selection on higher milk production and lower SCS is more practical, and selection index calculations demonstrate the response expected from different relative weights for MILK and SCS (Table 2.6). In the calculations, a correlation of 0.2 between MILK and SCS was assumed (Mrode and Swanson, 1996) and responses were calculated assuming selection on accurate breeding values for MILK and SCS (Cameron, 1997). The genetic correlations between CM caused by other streptococci and MILK and SCS were reduced by a factor 0.86 to stay within the parameter space. For overall CM the undesireable effect of selection for higher milk yield was counteracted when the relative weight to reduce SCS was twice the weight to increase yield (Table 2.6). For *Staph. aureus* and *Strep. dysgalactiae* the effect of selecting on higher milk yield was not counteracted by the simultaneous selection for lower SCS until most of the selection pressure was on reducing SCS.

Table 2.6 *Correlated responses (in genetic standard deviation units) from selection for an index with different relative weights for two 305d production traits (MILK and SCS), on MILK, SCS, overall clinical mastitis (CM) and pathogen-specific CM caused by Staphylococcus aureus (SAU), coagulase-negative staphylococci (CNS), Escherichia coli (ECO), Streptococcus dysgalactiae (SDY), Streptococcus uberis (SUB) and streptococci other than Strep. dysgalactiae and Strep. uberis (STR). A selection intensity of 1 genetic standard deviation is assumed*

Index weight		Expected responses in genetic standard deviations								
MILK	SCS	MILK	SCS	CM	SAU	CNS	ECO	SDY	SUB	STR
1.00	0.00	1.00	0.20	0.73	0.70	0.26	0.25	0.63	0.41	0.68
1.00	-0.20	0.98	0.00	0.67	0.66	0.16	0.13	0.63	0.32	0.52
1.00	-0.40	0.92	-0.20	0.57	0.59	0.04	0.00	0.61	0.22	0.34
1.00	-0.60	0.83	-0.38	0.47	0.51	-0.06	-0.12	0.57	0.12	0.16
1.00	-0.80	0.73	-0.52	0.36	0.42	-0.15	-0.22	0.52	0.03	0.00
1.00	-1.00	0.63	-0.63	0.27	0.34	-0.22	-0.30	0.47	-0.05	-0.14
0.80	-1.00	0.52	-0.73	0.17	0.25	-0.29	-0.37	0.40	-0.12	-0.27
0.60	-1.00	0.38	-0.83	0.05	0.14	-0.36	-0.45	0.32	-0.21	-0.42
0.40	-1.00	0.20	-0.92	-0.10	0.01	-0.44	-0.53	0.21	-0.31	-0.58
0.20	-1.00	0.00	-0.98	-0.25	-0.13	-0.50	-0.59	0.09	-0.40	-0.73
0.00	-1.00	-0.20	-1.00	-0.39	-0.27	-0.54	-0.63	-0.04	-0.47	-0.85

Philipsson et al. (1995) suggested that SCC should be decreased to the lowest possible value, at least within the range covered by the population mean and the genetic variance in their population. The estimated genetic correlation and calculated selection responses in the current study suggested that this also holds for the population in this study. For CNS and *E. coli* the effect of selecting on higher milk yield was already counteracted when the relative weight to reduce SCS was only half the weight to increase yield (Table 2.6). Nash et al. (2000) suggested as well that a reduction in, in particular, the number of cases of CM caused by CNS or environmental pathogens could indeed be expected from selection for lower SCS, in line with the fairly strong positive genetic correlations between SCS and CNS or environmental pathogens. This seems contradictory to Kehrli and Shuster (1994) who argued that cows with very low SCC might be more susceptible to CM, because their ability to respond to intramammary infections would be reduced. It also seems contradictory to the differences in occurrence of pathogen-specific CM at different BMSCC levels (Erskine et al., 1988; Hogan et al., 1989; Barkema et al., 1998). These authors all found a higher mean herd incidence of clinical *E. coli* mastitis in herds with low BMSCC (≤150,000 cells/ml) than in higher BMSCC herds. Also in our dataset, there is an indication of a higher risk of clinical *E. coli* mastitis with lower SCS, since the mean SCS is relatively low (2.42 and 2.77 over the first 150 and 305 days in milk, respectively (Table 2.3)), and the incidence of clinical *E. coli* mastitis is comparatively high (Table 2.4). However, neither the level of BMSCC nor the mean SCS reflects the SCS of individual cows, whereas genetic correlations do refer to the SCS of individual cows. When estimating the genetic correlations, a comparison is made between lactations with and without a case of pathogen-specific CM. Positive correlations demonstrate therefore that cows without CM are expected to have lower lactation SCS than cows with CM. This is something that holds for all pathogens and is confirmed by the phenotypic means of groups of lactations with and without a case of pathogen-specific CM. The largest difference for 305-day SCS was observed for cows with or without a case of clinical *Staph. aureus* mastitis during the lactation (4.15 *vs.* 2.72), and the smallest for cows with or without a case of clinical *E. coli* mastitis (3.18 *vs.* 2.76). The higher SCS for lactations with clinical *E. coli* mastitis explains why selection for lower SCS is expected to reduce clinical *E. coli* mastitis. These results, however, do not completely refute the theory that a risk of an undesireably low SCC exists. Since the mean SCS over a fixed time period (150 or 305 days) is used in the analyses, SCC from both before and after the case of pathogen-specific CM is used. Hence, it might still be that SCC is either very high or too low before a case of pathogen-specific CM. It requires other types of analyses to analyse this, where the mean SCC will be compared with the herd-test-days for SCC, used as separate traits. This might have the advantage that variation among test-days and variation in patterns of SCC could be used (Reents et al., 1995). Further analyses will be done to describe the effect of an infection on the individual cell counts.

Conclusions

Heritabilities for pathogen-specific CM were similar to that estimated for CM (0.02 to 0.10), and repeatabilities were low (0.10 to 0.14). Genetic correlations were quite strongly positive for pathogen-specific CM with milk yield and SCS, but standard errors were large. Still, the genetic correlations with milk yield and SCS seemed to differ according to pathogen. In particular, selection for higher milk yield alone might cause less increase in the incidence rate of clinical *E. coli* mastitis than in the incidence rate of clinical *Strep. dysgalactiae* mastitis. Based on the calculated selection indices, simultaneous selection for lower somatic cell counts would be more effective in counteracting the undesireable effect on clinical *E. coli* mastitis, but of less benefit in the case of clinical *Strep. dysgalactiae* mastitis.

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Chapter 3

Effect of pathogen-specific clinical mastitis on lactation curves for somatic cell count

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Abstract

Data from 274 Dutch herds recording clinical mastitis (CM) over an 18-month period were used to investigate the effect of pathogen-specific CM on the lactation curve for somatic cell count (SCC). Analysed pathogens were *Staphylococcus aureus*, coagulase-negative staphylococci, *Escherichia coli*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, other streptococci, and the culture-negative samples. The dataset contained 178,754 test-day records on SCC, recorded in 26,411 lactations of 21,525 cows of different parities. In lactations without both clinical and subclinical mastitis, SCC was high shortly after parturition, decreased to a minimum at 50 days in milk, and increased slowly toward the end of the lactation. Effects of CM on lactation curves for SCC differed among the pathogens isolated. Before a case of clinical *E. coli* mastitis occurred, SCC was close to the SCC of lactations without both clinical and subclinical mastitis, and after the case of CM had occurred, SCC returned rather quickly to a low level again. Similar curves were found for lactations with cases of CM associated with culture-negative samples. Before a case of clinical *Staph. aureus* mastitis occurred, average SCC was already high, and it remained high after the occurrence. Effects of CM associated with *Strep. dysgalactiae*, *Strep. uberis,* and other streptococci on the lactation curve for SCC were comparable. They showed a continuous increase in SCC until the case of pathogen-specific CM occurred, and afterwards SCC stayed on a higher level. Using SCC test-day records, these typical characteristics of each pathogen may be used to find more effective indicators of CM.

Keywords Somatic cell count, Lactation curve, Clinical mastitis, Pathogens

Introduction

In mastitis-control programs and for genetic improvement, somatic cell count (SCC) is often used to monitor udder health. For example, breeding values for lactation-average SCC are used in selection to decrease the prevalence of subclinical mastitis (SCM) and the incidence of clinical mastitis (CM) (Mrode and Swanson, 1996). Average lactation values of SCC are used in selection programs, and variation during lactation among test-day records for SCC is most often ignored (Reents et al., 1995). Variation during lactation can be taken into account by using a test-day model, and it is expected that the longitudinal SCC data provide additional information about the pathogens involved in cases of CM, for example, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*.

Suggestions that longitudinal test-day records for SCC might provide information about pathogens involved is based on comparison at herd level and on cows with experimentally induced pathogen-specific CM. When grouping dairy herds on their bulk milk SCC (BMSCC), the incidence of clinical *Staph. aureus* and *Strep. dysgalactiae* mastitis was higher in herds with high BMSCC than in herds with low BMSCC. The opposite was true for the incidence of clinical *E. coli* mastitis or of CM associated with culture-negative samples (Eberhart et al., 1982; Erskine et al., 1988; Hogan et al., 1989; Sischo et al., 1993; Barkema et al., 1998). These results may indicate that pathogens are associated with either different baseline levels for SCC or different duration of cases of CM, as both of them may affect BMSCC. Experiments that induced CM showed that two days after inoculation with *E. coli* SCC peaks, and the preinfection value is approached within three to four weeks again (Erskine et al., 1992; Pyörälä et al., 1994). However, within 24h after the inoculation with *Staph. aureus*, SCC increases and remains high for at least 48 days (Shoshani et al., 2000). These studies support that pathogen-specific effects on lactation curves for SCC might exist for both level and duration of SCC increases.

In two studies, the effect of infection status on cow SCC was investigated under practical circumstances (Sheldrake et al., 1983; Schepers et al., 1997). Sheldrake et al. (1983) compared lactation curves for SCC of quarters free from CM with lactation curves for SCC of quarters with clinical *Staph. aureus*, coagulase-negative staphylococci (CNS), and *Corynebacterium bovis* mastitis. Quarters with clinical *Staph. aureus* mastitis showed a considerable increase in SCC and quarters with known infection had higher SCC than quarters free from CM. Schepers et al. (1997) showed how different pathogens caused a different increase in quarter SCC. The largest increase was found for *Staph. aureus* and the smallest for *Corynebacterium bovis*. Unfortunately, patterns of SCC before and after a case of pathogen-specific CM were not included in these studies, and specific information on the number of days in milk (DIM) when CM occurred was not available. However, the patterns and the DIM of occurrence of CM might be useful in distinguishing between pathogens.

The overall objective in this study was to investigate pathogen-specific effects on SCC during lactation. Most published lactation curves for SCC are determined from a dataset containing both lactations with and without CM (Wiggans and Shook, 1987; Schutz et al., 1990; Weller et al., 1992). Therefore, the first objective was to estimate the effect of CM and SCM on the lactation curve. The second objective was to analyse the pattern of SCC before and after a case of pathogen-specific CM, relative to the lactation curve for lactations without both CM and SCM.

Material and Methods

Herds. Records on CM were available from December 1992 until June 1994 on 274 Dutch farms (Barkema et al., 1998). Lactating cows were housed in free-stall barns, and milking parlours were double-herringbone or two-sided open tandem. Herds participated in the milk recording system, and annual milk production quotas were between 300,000 and 900,000 kg. The national milk recording system (NRS, Arnhem, The Netherlands) provided information from the three or four-weekly milk recordings. A record included national cow identification, breed, date of milk recording, date of calving, date of drying off, test-day milk yields (kg of milk, fat, and protein) and SCC (cells/ml). The breed of the cow was divided in maximal three subclasses. The main breeds were Holstein-Friesian (HF), Dutch-Friesian and Meuse-Rhine-Yssel.

Bacteriological sampling. During the study, farmers were asked to collect milk samples from every quarter that they observed with CM. The aseptic sampling procedures are described by Barkema et al. (1998). Data collection of cases of CM depends heavily on the willingness of the farmers to collect milk samples, and the farmers were continually encouraged to avoid the potential bias in reporting the cases of CM, as described in Barkema et al. (1998). The samples were stored in a freezer at the farm (at approximately -20°C), and were collected for bacteriological examination at intervals of six to eight weeks. Bacteriological culturing of milk samples was performed according to the standards of the National Mastitis Council (Harmon et al., 1990). From each milk sample 0.01 ml was cultured, and in each culture the number of colony-forming units of each of the bacterial species was counted. Collected data contained information on the national cow identification, date of occurrence, infected quarter, and the outcome of the bacteriological culturing of the milk samples. For the analyses, only the first case of CM in each lactation was considered, and seven groups of pathogens were defined based on the incidence in the data; i.e. *Staph. aureus*, CNS, *E. coli*, *Strep. dysgalactiae*, *Strep. uberis*, streptococci other

than *Strep. dysgalactiae* and *Strep. uberis,* and culture-negative samples. Cases of CM associated with any other pathogen or mixed cultures were grouped together.

Data selection. Originally, phenotypic records on CM and bacteriological characterisation were available on 49,529 lactations that had been recorded for at least one day during the study. Selection criteria described by De Haas et al. (2002) reduced the dataset to 47,563 lactations. For the present study, only lactations that had been recorded from calving onward were included in the dataset, to ensure that no previous cases of CM had occurred within the lactation. This criterion reduced the dataset to 26,427 lactations with 178,986 test-days recorded before 308 DIM. Herd-test-date (HTD) classes with fewer than two observations for SCC were deleted from the dataset, and, therefore, the final dataset consisted of 26,411 lactations from 21,525 cows (= dataset 1). In these lactations 178,754 test-days with SCC were recorded, and 3,781 first cases of CM were observed.

Lactation curves. Three different lactation curves for SCC were determined, based on (1) the test-day records from all available lactations (= dataset 1), (2) only the test-day records from lactations without a case of CM (= dataset 2), and (3) only the test-day records from lactations without a case of CM and with one or fewer measure of SCC above 250,000 cells/ml during the first 308 DIM (= dataset 3). Dataset 3 was created to elucidate most of the effects of SCM on the lactation curve for SCC. The threshold of 250,000 cells/ml was chosen based on findings of Dohoo and Leslie (1991). For any threshold between 200,000 and 300,000 cells/ml, they found a reasonably high specificity and quite low proportions of cows that were not at risk of developing a new infection, but which met the test criterion they set. Dataset 2 and 3 contained 151,156 test-day records of SCC in 22,630 lactations, and 117,598 test-day records of SCC in 18,438 lactations, respectively. The lactation curves were determined for heifers and multiparous cows separately by fitting an interaction with parity in the statistical model. This separation was made because multiparous cows might have had CM or SCM in a previous lactation, and there is evidence that these curves differ (Schutz et al., 1990; Weller et al., 1992; Schepers et al., 1997).

Effects of infection status. Two steps were involved in investigating the effect of a case of pathogen-specific CM on the lactation curve for SCC. First, SCC was expressed relative to SCC in lactations without both CM and SCM. Therefore, the SCC on each DIM for the lactations without both CM and SCM was estimated using the lactations included in dataset 3. These estimates were subtracted from the SCC recorded on the test-days included

in dataset 1, matching on DIM. The procedure was carried out separately for heifers and multiparous cows, and this calculated value for SCC is referred to as the corrected SCC (CSCC). Secondly, the DIM of each CSCC record was expressed relative to the DIM of the first recorded case of pathogen-specific CM (DIM_CM). The full range of 308 days before and after CM was used in the analyses, because the spline function estimated CSCC for every DIM_CM recorded in the dataset. Lactations with CM occurring late in the lactation provided information on SCC before a case of CM, and lactations with CM occurring early in the lactation provided information on SCC after a case of CM. Between 69 and 133 CSCC records were available on each DIM_CM around the day of occurrence of CM (-15 to 15 DIM_CM). Curves for CSCC were estimated for heifers and multiparous cow separately by fitting an interaction with parity in the statistical model.

Statistical analyses. Three lactation curves for SCC as a function of DIM, and the eight CSCC curves as a function of DIM_CM, were estimated for both heifers and multiparous cows, using the spline function in AS-REML (Gilmour et al., 2001). Usually, a spline function is used for smoothing data points, and the function allows maximum flexibility and assumes no prescribed curvature. The spline function was chosen because of the expected sudden changes in SCC around a case of CM and the large amount of data available. A cubic spline is a piecewise cubic function that is constrained so that the function and its first two derivatives are continuous at the breakpoints (knots) between one segment and the next.

The AS-REML program fits conventional smoothing splines, but the number and location of the knots was chosen in advance (White et al., 1999). For the curves of SCC as a function of DIM and of CSCC as a function of DIM_CM, 22 and 45 knots were set, respectively. For SCC the knots were set closer to each other at the beginning of the lactation, because it was expected that SCC would change rapidly in the first 50 DIM but more smoothly after that toward the end of the lactation. The knots were set at 2-day intervals from 0 to 10 DIM, 5-day intervals from 10 until 30 DIM, 10-day intervals from 30 to 50 DIM, and 25-day intervals from 50 DIM toward the end of the lactation. For CSCC, the knots were symmetrically distributed before and after the case of CM. In the days around the case of CM, it was expected that large changes in CSCC would occur, so the knots were set closer to each other in this period to be able to capture these changes. The knots were fixed on the day of occurrence (day 0), and at one and two days before and after DIM_CM to be able to estimate the peak of CSCC. Intervals of five days were set from 5 to 20 days before and after DIM_CM, followed by 10-day intervals until 100 days before and after DIM_CM and 25-day intervals until 300 days before and after DIM_CM.

The spline function for DIM was nested within each parity class (n = 2) to be able to estimate lactation curves for SCC for heifers and multiparous cows separately. Using all data

simultaneously, rather than splitting the data, allowed more accurate adjustment for HTD. The 'predict' statement in AS-REML was used to estimate SCC on each DIM, and the lactation curves for SCC were created by plotting these estimates for SCC per DIM, for both heifers and multiparous cows. For CSCC curves, the spline function for DIM_CM was nested within each combination of parity (n = 2) and pathogen (n = 9; one class for lactations without CM, seven classes for defined pathogens, and one class grouping other pathogens and mixed cultures). The CSCC on each DIM_CM was estimated by the spline function, for each combination of parity and pathogen. The CSCC curves were plotted using the interval between 150 days before until 250 days after the case of CM, because there were limited records outside this interval (i.e., fewer than 15 test-day recordings on each DIM_CM).

Additional fixed effects in the model were the fraction of HF-genes (with 9 classes, for 0, 1/8, …, 8/8) as a linear and quadratic regression, age at calving as a linear and quadratic regression, and HTD (with 4,750 levels).

Results

Lactation curves for SCC. The lactation curve for SCC based on all available lactations for heifers (= dataset 1) was high shortly after parturition (370,000 cells/ml), decreased to a minimum of 98,000 cells/ml around 50 DIM, and increased slowly toward 139,000 cells/ml at the end of the lactation (Figure 3.1a). A similar pattern was found for lactations without a case of CM (= dataset 2), but SCC was slightly lower throughout the lactation (10,000 to 30,000 cells/ml). Estimated SCC for lactations without both CM and SCM (= dataset 3) was generally low compared with all lactations and the lactations without a case of CM (= dataset 1 and 2, respectively). Although shortly after parturition, the estimated SCC was approximately 50,000 cells/ml higher in dataset 3 than in datasets 1 and 2. But at 50 DIM, SCC had already decreased to 57,000 cells/ml, and it increased from then on to 82,000 cells/ml at the end of the lactation.

Multiparous cows had generally higher SCC on each DIM than heifers. The difference ranged from 20,000 cells/ml at the start to 145,000 cells/ml at the end of the lactation for dataset 1 and from 10,000 to 125,000 cells/ml for dataset 2 (Figure 3.1a – 3.1b). For the heifers in dataset 3, the estimated SCC until 38 DIM was high compared with those for multiparous cows, but after 38 DIM it was the opposite. For heifers, the three lactation curves based on datasets 1, 2, and 3 were closer to each other, than for multiparous cows (Figure 3.1a *vs.* Figure 3.1b). For instance, the difference between the lactation curves based on datasets 2 and 3 was, on average, 37,000 cells/ml for heifers, whereas for multiparous cows this difference was, on average, 105,000 cells/ml.

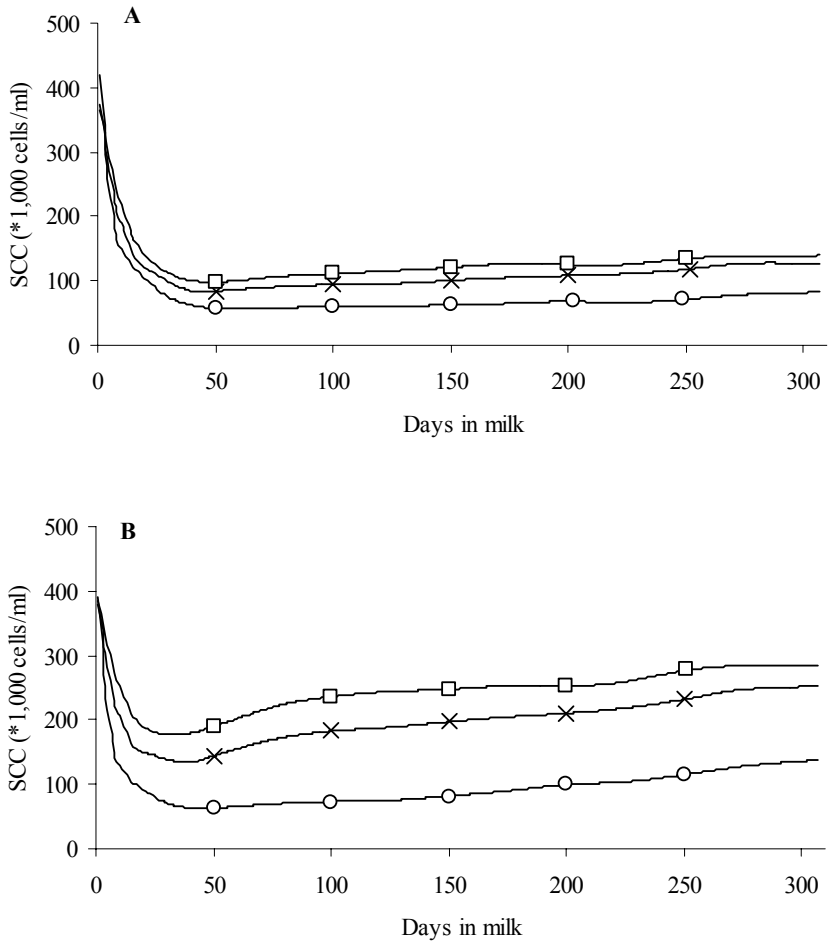

Figure 3.1a – 3.1b *Somatic cell count during the lactation, plotted using the estimates from the spline function for all available lactations* (□)*, all lactations without a case of CM* (×)*, and all lactations without both clinical and subclinical mastitis* (○) *for (a) heifers and (b) multiparous cows, respectively. On average, the standard error from each estimated SCC in the first 7 DIM was approximately 25,000 cells/ml, and it was 10,000 cells/ml in the rest of the lactation, for all curves.*

Somatic cell count rose with increasing age at calving and HF-percentage. The linear and quadratic regression coefficients for age at calving indicated a nearly linear increase of SCC between 500 and 3500 days, with differences close to 385,000, 315,000 and 80,000 cells/ml in datasets 1, 2, and 3, respectively (after adjusting for parity differences). The regression coefficients for HF-percentage indicated a nearly linear increase of SCC as well between 0 and 100% HF, with differences between the two extremes close to 41,000, 26,000 and 7,000 cells/ml in datasets 1, 2, and 3, respectively.

Distribution of cases of CM. The distribution of pathogens involved in cases of CM is shown in Table 3.1. This distribution was nearly the same in heifers and multiparous cows. In heifers, CNS and *Strep. dysgalactiae* were more often isolated than in multiparous cows. In multiparous cows, *E. coli* was the most isolated pathogen.

Table 3.1 also shows the distribution of the DIM of occurrence of pathogen-specific CM. Clinical mastitis associated with all pathogens, except *E. coli*, occurred earlier in heifers than in multiparous cows. The mean DIM for clinical *E. coli* mastitis was similar for heifers and for multiparous cows (82 and 80 DIM, respectively). In the first week after calving, 25% of the cases of CM associated with all pathogens except *E. coli* had occurred in heifers. Half of the cases of CM caused by CNS, *Strep. dysgalactiae*, *Strep. uberis* and other streptococci had occurred in the first 14 DIM. In the first 90 DIM, 75% of the cases of CM caused by *Strep. dysgalactiae*, *Strep. uberis* and other streptococci had occurred in the heifers.

There tended to be a difference between heifers and multiparous cows in the pathogens causing CM in late lactation. In heifers, clinical *E. coli* and *Strep. uberis* mastitis tended to occur until late in the lactation, whereas in multiparous cows, cases of CM associated with *Staph. aureus* and streptococci other than *Strep. dysgalactiae* and *Strep. uberis* tended to occur until late in the lactation.

The distribution of lactations with CM per class of HF-percentage is shown in Table 3.2. The percentage of lactations with CM ranged from 12.9 to 16.6%, except in the lactations of animals with 1/8 HF (i.e. 33.3%).

Table 3.1 *Number of cases of pathogen-specific clinical mastitis (CM) (n), relative percentage (%), the mean DIM, and the number of days in milk (DIM) that 10%, 25%, 50%, 75% and 90% of the cases of pathogen-specific CM had occurred, for heifers and multiparous cows, separately*

			DIM					
	n	%	Mean	10%	25%	50%	75%	90%
Heifers								
Staphylococcus aureus	85	11.7	65	0	2	35	106	153
Coagulase-negative staphylococci	53	7.3	52	0	1	6	101	163
Escherichia coli	121	16.7	82	1	12	56	118	206
Streptococcus dysgalactiae	74	10.2	44	0	1	10	56	145
Streptococcus uberis	34	4.7	66	2	6	14	86	248
Other streptococci	43	5.9	48	0	3	14	71	165
Culture-negative samples	124	17.1	72	2	6	38	120	187
Multiparous cows								
Staphylococcus aureus	406	13.3	80	2	18	64	120	195
Coagulase-negaive staphylococci	108	3.5	85	2	26	74	125	176
Escherichia coli	658	21.5	80	3	24	61	123	179
Streptococcus dysgalactiae	204	6.7	73	2	23	59	98	176
Streptococcus uberis	165	5.4	79	2	16	60	119	182
Other streptococci	173	5.7	85	3	13	68	130	203
Culture-negative samples	489	16.0	78	2	10	55	120	201

Table 3.2 *The number of lactations (# lact) and the number of lactations with clinical mastitis (# lact CM1) for each fraction of Holstein-Friesian genes*

	# lact	# lact CM1
Holstein-Friesian		
0	1,109	170
1/8	3	1
2/8	131	20
3/8	634	83
4/8	3,498	579
5/8	1,286	193
6/8	9,060	1,329
7/8	8,243	1,061
8/8	2,447	345

Effect of pathogen-specific CM on SCC. In general, the mean CSCC before CM was higher for multiparous cows than for heifers, except before CM associated with CNS or *Strep. dysgalactiae* (Table 3.3). After a case of CM, the mean CSCC for heifers was always lower than for multiparous cows, but around a case of CM the CSCC for heifers and multiparous cows were similar. The average standard error over all DIM_CM was rather large, so the CSCC curves do not seem to differ between heifers and older cows.

Effects of pathogens differed for CSCC. Before a case of clinical *E. coli* mastitis CSCC was close to zero (Figure 3.2b), that is, close to SCC of lactations without both CM and SCM. However, 100 days before a case of clinical *Staph. aureus* mastitis occurred, CSCC was already on a higher level, that is, 200,000 and 325,000 cells/ml for heifers and multiparous cows, respectively (Figure 3.2a). After a case of clinical *E. coli* mastitis had occurred, CSCC returned rather quickly to a low level again (100,000 and 200,000 cells/ml for heifers and multiparous cows, respectively), whereas CSCC remained high after a case of clinical *Staph. aureus* mastitis, that is, 200,000 and 450,000 cells/ml, respectively (Table 3.3). The CSCC curves before and after cases of CM associated with *Strep. dysgalactiae* and *Strep. uberis* were similar to each other (Figure 3.2c – 3.2d). A continual increase in CSCC was found during the days before the cases of CM occurred. After the cases of CM had occurred, CSCC tended to stay high for multiparous cows, whereas for heifers CSCC decreased slowly to a lower level. Similar CSCC curves were also found before and after a case of CM associated with streptococci other than *Strep. dysgalactiae* and *Strep. uberis* (results not shown). For heifers, CSCC lowered to a level close to SCC of lactations without both CM and

SCM after a case of CM associated with streptococci other than *Strep. dysgalactiae* and *Strep. uberis* (Table 3.3). The CSCC curves before and after occurrence of cases of CM with culture-negative samples occurred were comparable to the curves before and after clinical *E. coli* mastitis (results not shown).

Table 3.3 *The estimates for corrected somatic cell count (CSCC (* 1,000 cells/ml)) and the standard error (se) averaged from 150 to 21 days before a case of pathogen-specific clinical mastitis (before CM), from 20 days before to 20 days after a case of pathogen-specific clinical mastitis (around CM), and from 21 to 250 days after a case of pathogen-specific clinical mastitis (after CM), for heifers and multiparous cows, separately*

	Before CM		Around CM		After CM	
	CSCC	se	CSCC	se	CSCC	se
Heifers						
Staphylococcus aureus	198	57	559	44	213	49
Coagulase-negative staphylococci	217	72	655	56	192	62
Escherichia coli	9	46	423	37	105	42
Streptococcus dysgalactiae	241	72	674	57	169	61
Streptococcus uberis	171	69	679	50	172	64
Other streptococci	146	82	517	67	51	74
Culture-negative samples	46	47	366	37	141	41
Multiparous cows						
Staphylococcus aureus	356	34	748	30	460	30
Coagulase-negative staphylococci	164	49	682	39	352	45
Escherichia coli	84	30	546	28	203	26
Streptococcus dysgalactiae	226	46	748	37	285	38
Streptococcus uberis	212	46	867	38	330	41
Other streptococci	227	42	748	35	306	40
Culture-negative samples	139	32	431	29	214	28

Discussion

Effect of parity and stage of lactation. The level of SCC has been reported to be influenced by parity (Blackburn, 1966; Lindström et al., 1981), stage of lactation (Blackburn, 1966; Bodoh et al., 1976), season (Bodoh et al., 1976; Kramer et al., 1980), and environmental and management factors (Bodoh et al., 1976). In the current study, we have corrected for environmental and management factors by adjusting for HTD in the statistical model. The effects of parity and stage of lactation have been considered and were found to be similar to what other studies have reported (Emanuelson and Persson, 1984; Wiggans and Shook, 1987; Schutz et al., 1990; Schepers et al., 1997). All these studies concluded that the increase in SCC toward the end of the lactation was not as great for heifers as for multiparous cows. These results might be affected by infection status, but Schepers et al. (1997) analysed SCC measures of uninfected quarters. They also found that the increase in the logarithm of SCC toward the end of the lactation was more pronounced for multiparous cows and that the lactation curve for SCC for heifers was relatively flat.

The different shapes of the lactation curves with increasing parities indicate that SCC early and late in life may be different traits, which supports the conclusions of Coffey et al. (1985). They suggested that different mechanisms of defence against mammary infections are of primary importance at different ages and that those most important at older ages are genetically more variable.

Standard lactation curve for SCC. In the present study, the effect of a case of naturally occurring pathogen-specific CM on the lactation curve for SCC was estimated. Records for SCC were preadjusted for the lactation curve based on lactations without CM and with maximum one SCC test-day record above 250,000 cells/ml. We assumed that these criteria excluded lactations with both CM and SCM from dataset 1, which is probably not perfect. First, data from cows directly culled after CM, and therefore having only one test-day record above 250,000 cells/ml were still included in dataset 3. Also, with the threshold of 250,000 cells/ml, the presence of pathogens in the udder might not have completely been excluded. For example, Laevens et al. (1997) calculated geometric mean SCC of bacteriological negative cows at various stages of lactation. Cows were all under 120,000 cells/ml, with a mean of 50,000 cells/ml. Furthermore, Huxley et al. (2001) had to lower the threshold to 25,000 cells/ml to have no quarter infected with a major pathogen.

Setting a more stringent criterion, however, was not expected to have a large influence on the lactation curves for SCC, except for the mean. Therefore, results in this study are not expected to be influenced by the criterion of 250,000 cells/ml, as these are all estimated relative to the same mean.

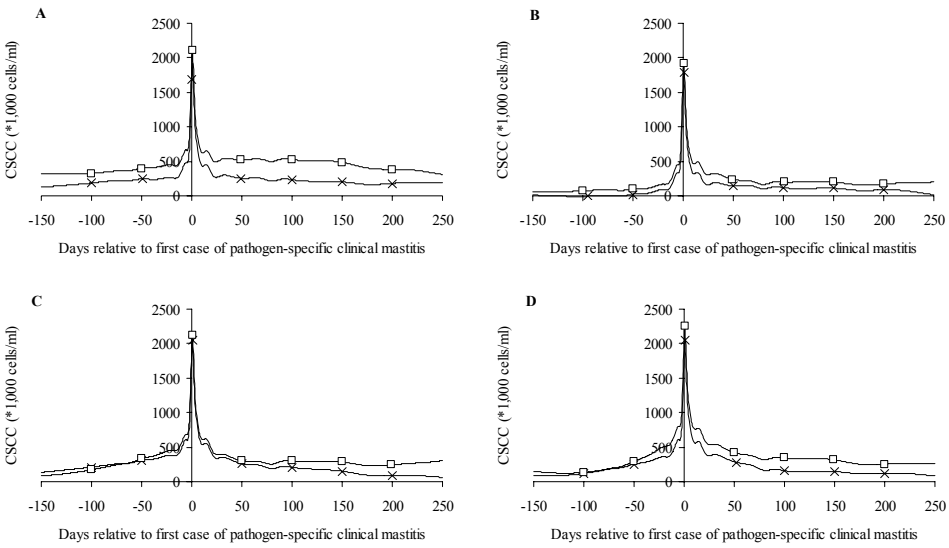

Figure 3.2a – 3.2d *The pattern of SCC before and after first cases of clinical mastitis associated with (a) Staphylococcus aureus, (b) Escherichia coli, (c) Streptococcus dysgalactiae, and (d) Streptococcus uberis, relative to the SCC of lactations without both clinical and subclinical mastitis (CSCC), for heifers (×) and multiparous cows* (□).

Pathogen-specific effects on CSCC. The pathogen-specific effects on the CSCC curves showed clearly differential effects for *Staph. aureus* and *E. coli*. Before a case of clinical *Staph. aureus* mastitis occurred, CSCC was high, higher than SCC in lactations without both CM and SCM, for both heifers and multiparous cows. This suggests that the pathogen is subclinically present for some time already before clinical symptoms are observed. *Staphylococcus aureus* is known to cause chronic and subclinical mastitis, with periodic clinical episodes (Harmon, 1994). That might explain why CSCC stays high after the case of clinical *Staph. aureus* mastitis and why it takes long to stabilise on the lowest level, especially for multiparous cows.

Before a case of clinical *E. coli* mastitis, CSCC was low, that is, close to the level in lactations without both CM and SCM. After the case of clinical *E. coli* mastitis, CSCC decreased rather rapidly to a level that was only slightly higher than the preinfection level, which has been reported by Erskine et al. (1992) and Pyörälä et al. (1994) as well. Cows

with clinical *E. coli* mastitis have more systemic clinical signs than cows with clinical *Staph. aureus*, *Strep. dysgalactiae* or *Strep. uberis* mastitis (Miltenburg et al., 1996). According to Harmon (1994) approximately 70 to 80% of the intramammary *E. coli* infections become clinical. Smith et al. (1985) studied the rate of intramammary infections (IMI) caused by environmental streptococci (e.g. *Strep. dysgalactiae* and *Strep. uberis*), and, on average, 53% of all streptococcal IMI was associated with clinical symptoms.

Pathogen-specific effects on a lactation curve for SCC are a combination of the standard lactation curve and the CSCC curves. To illustrate this, the estimates for CSCC (Figure 3.2a – 3.2d) were added to the estimates for SCC in dataset 3 (Figure 3.1a – 3.1b), with the assumption that the case of pathogen-specific CM occurred on the median DIM (Figure 3.3a – 3.3b). For multiparous cows, 50% of all cases of clinical *Staph. aureus*, *E. coli*, *Strep. dysgalactiae* and *Strep. uberis* mastitis had occurred around 60 DIM, whereas for heifers the medians ranged from 10 to 56 DIM (Table 3.1). The underlying assumption is that the CSCC curves do not depend on the DIM of occurrence of pathogen-specific CM, and are, therefore, the same before and after all cases of CM. However, with the median DIM so close to calving, there is little information available on SCC before the cases of CM, and the CSCC curves will be determined predominantly by cases of CM occurring later during the lactation. The lactation curves for heifers with a case of clinical *Staph. aureus*, *Strep. dysgalactiae* and *Strep. uberis* mastitis starts at a high level immediately after calving, and this suggests that the pathogens are already present at calving. Matthews et al. (1992) reported a rather high prevalence of *Staph. aureus* in heifers before calving (± 7%), and a higher prevalence of *Staph. aureus* in heifers than in multiparous cows at parturition.

Kehrli and Shuster (1994) argued that cows with very low SCC might be more susceptible to CM, because their ability to respond to IMI would be reduced. However, the results in this study do not support that cows with low SCC during early lactation are more susceptible for CM. For none of the pathogens was SCC before a case of CM below the level of lactations without both CM and SCM. For heifers, the SCC was virtually the same before a case of clinical *E. coli* mastitis, and for multiparous cows slightly higher. This, together with the increase in SCC both before and after a case of CM associated with most pathogens, suggests that avoiding high SCC is an important tool to reduce CM.

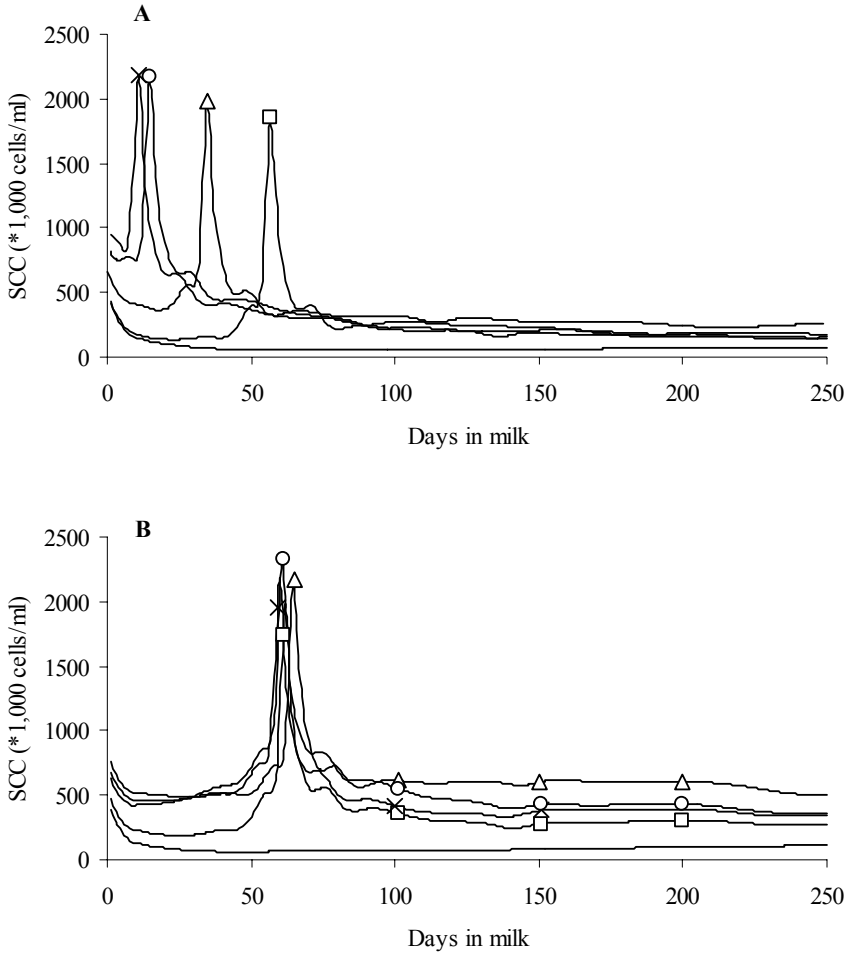

Figure 3.3a – 3.3b *A comparison of lactation curves for SCC for lactations without both clinical and subclinical mastitis (solid line) and lactations with clinical Staphylococcus aureus mastitis* (Δ)*, clinical Escherichia coli mastitis* (□)*, clinical Streptococcus dysgalactiae mastitis* (×) *and clinical Streptococcus uberis mastitis* (○) *occurring on the median DIM for (a) heifers and (b) multiparous cows, respectively.*

Use of test-day records. Heuven (1987) analysed test-day records of SCC to predict the presence of pathogens and developed a method to identify abnormal observations of SCC, in order to exclude them from the dataset. An observation was considered to be abnormal on the basis of its deviation from the normal lactation curve. While using this exclusion method, he concluded that cows with high deviations from the normal lactation curve were more likely to be treated for CM. Besides the SCC test-day records, other sources could provide additional information for an accurate prediction of the pathogen that is involved. This additional information can, for example, be related to (1) the cow, that is, lactation stage and parity, (2) the presence of general clinical signs, that is, body temperature and condition of the quarter (Green, 1998), and (3) the occurrence of another case of pathogen-specific CM earlier in the lactation (Lam et al., 1997; Barkema et al., 1999). However, the advantage of SCC is that routinely recorded data can be used on a large scale.

By using the lactation-average SCC, the dynamics in SCC during the lactation are ignored, whereas a priori we expected that these might be informative for the susceptibility to CM. This is important because different pathogens affect SCC differently and because it was demonstrated that the risk for CM changes during the lactation (Barkema et al., 1998). The results in this study confirm that SCC curves and the DIM of occurrence differ for pathogens (Figure 3.3a – 3.3b). By applying SCC test-day records more effectively, the typical characteristics of pathogens could be used to predict the pathogen involved in a case of CM. Further analyses will be done on a more effective use of SCC test-day records, by replacing lactation-average SCC by newly defined traits for SCC, depending on the lactation curve for SCC. These new traits for SCC should distinguish between pathogen-specific effects on the lactation curve for SCC.

Conclusions

The lactation curve for SCC started off high shortly after parturition, decreased to a nadir around 50 DIM, and increased slowly toward the end of the lactation. Effect of CM on the lactation curve for SCC was large but differed per pathogen. Somatic cell count always remained elevated after a case of pathogen-specific CM, although the effect was smaller with larger intervals between the occurrence of CM and the day of sampling. After a case of clinical *E. coli* mastitis, SCC approached the preinfection value after 50 DIM, whereas SCC remained high after clinical *Staph. aureus* mastitis. Increased SCC was shown before cases of CM associated with *Staph. aureus*, *Strep. dysgalactiae*, *Strep. uberis,* and other streptococci, but SCC was low before the occurrence of clinical *E. coli* mastitis. These typical characteristics of pathogens might be useful when, instead of lactation-average SCC, SCC test-day records will be used in mastitis control programs or for genetic improvement. Test-day SCC may be used as indicators of the pathogen involved in a case of CM.

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Chapter 4

Associations between pathogen-specific clinical mastitis and somatic cell count patterns

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Abstract

Associations were estimated between pathogen-specific cases of clinical mastitis (CM) and patterns in somatic cell count (SCC) based on deviations from the typical curve for SCC during lactation, and compared with associations between pathogen-specific CM and lactation-average SCC. Data from 274 Dutch herds recording CM over an 18-month period were used. Pathogens found were *Staphylococcus aureus*, coagulase-negative staphylococci, *Escherichia coli*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, streptococci other than *Strep. dysgalactiae* and *Strep. uberis* and culture-negative samples. The dataset contained 245,595 test-day records on SCC, recorded in 24,012 lactations of 19,733 cows of different parities. Pattern definitions were based on three or five consecutive test-day records. The patterns differentiated between a short or longer period of increased SCC, and also between lactations with and without recovery. Logistic regression was applied to identify associations between presence of patterns and occurrence of pathogens. Presence of SCC patterns in a lactation predicts occurrence of overall CM in that lactation equally or even more accurate than presence of lactation-average SCC of more than 200,000 cells/ml. Patterns can also distinguish between chances of risk for specific mastitis-causing pathogens. Clinical *E. coli* mastitis was significantly associated with the presence of a short peak in SCC ($p < 0.05$), whereas *Staph. aureus* was associated with long increased SCC. *Streptococcus dysgalactiae* was not strongly associated with any of the defined patterns of peaks in SCC, and no single unambiguous pattern was found for *Strep. uberis*.

Keywords Somatic cell count, Patterns, Lactation curve, Clinical mastitis, Pathogens

Introduction

Average lactation values of somatic cell count (SCC) are generally used in mastitis control programs and for genetic improvement of udder health. However, these average values ignore variation in SCC during lactation. Curves for SCC during lactation decline to nadir before 60 days in milk (DIM), and increase during the remainder lactation (Wiggans and Shook, 1987; Schutz et al., 1990; Weller et al., 1992; De Haas et al., 2002a). Clinical and subclinical mastitis can cause deviations from this typical curve of SCC, and it has been shown that specific pathogens involved in cases of clinical mastitis (CM) affect the curve differentially (De Haas et al., 2002a).

Using test-day records of SCC instead of lactation-average SCC increases the possibilities to identify deviations from the typical curve of SCC during lactation. These deviations might characterise mastitis-causing pathogens. If so, analysing test-day records of SCC can be more useful in attempting to decrease the prevalence of subclinical mastitis (SCM) and the incidence of CM, than the lactation-average SCC. An effective use of SCC test-day records might be achieved by defining patterns of peaks in SCC during the lactation. If these patterns of peaks in SCC provide information on (1) the pathogen-distribution on a farm, (2) the success of therapy, and (3) the status of immunity of the cows, these patterns might be better tools to use in mastitis control programs or for genetic selection programs. Management can then be directed specifically on lowering the incidence of pathogen-specific CM, or shortening the duration of infection. Furthermore, a decreased genetic susceptibility for the full scope of mastitis-causing pathogens might be accomplished, which cannot completely be achieved by genetic selection on lower lactation-average SCC (Nash et al., 2000; De Haas et al., 2002b). Eventually, this all helps to limit the losses due to CM.

Before the patterns of peaks in SCC can be used for health management on farms or for genetics, we first have to find out if patterns of peaks in SCC do differ from the lactation-average SCC. Therefore, the overall objectives of this study were: (1) to define several patterns of peaks in SCC, and then, (2) to test their sensitivity and specificity, and, (3) to estimate their associations with occurrence of pathogen-specific cases of CM, also in comparison with the lactation-average SCC.

Material and Methods

Herds. Records on CM were available from a longitudinal prospective cohort study from December 1992 till June 1994 on 274 Dutch farms (Barkema et al., 1998). Lactating cows were housed in free-stall barns and milking parlours were double herringbone or two-sided

open tandem shape. Herds participated in a milk recording system, and annual milk production quotas were between 300,000 and 900,000 kg. The national milk recording system (NRS, Arnhem, The Netherlands) provided information from a three- or four-weekly milk recording system. A record included national cow identification, breed, date of milk recording, date of calving, date of drying off, test-day milk yields (kilograms milk, fat and protein) and SCC (cells per ml). The main breeds were Holstein-Friesian (HF), Dutch-Friesian and Meuse-Rhine-Yssel.

Sampling. Epidemiological studies often depend on farmer-observed disease incidence, introducing a possible bias caused by variation between farmers in diagnostic criteria. Farmer-diagnosed CM was analysed in this study. Lam et al. (1993) concluded that the diagnostic capability of farmers does not have a negative influence on the validity of farmer-diagnosed CM. Farmer-diagnosed CM, therefore, seems to be a useful tool in epidemiological studies on CM.

Selection of herds and aseptic sampling procedures have been described previously (Barkema et al., 1998). Data collection of milk samples of quarters with CM depended heavily on the willingness of farmers. Therefore, farmers were continuously encouraged, as described by Barkema et al. (1998). During the study period, farmers took milk samples from all quarters that, in their opinion, had clinical signs of mastitis. Samples were stored in a freezer at the farm (at approximately -20°C) and were collected for bacteriological examination at intervals of six to eight weeks. Bacteriological culturing of milk samples was performed according to the standards of the National Mastitis Council (Harmon et al., 1990). Briefly, 0.01 ml was cultured, and for each culture, the number of colony-forming units of each of the bacterial species was counted. Collected data contained information on national cow identification number, date of mastitis occurrence, infected quarter, and result of bacteriological culturing of milk sample. Seven groups of pathogens were defined based on their incidence in the data: *Staphylococcus aureus*, coagulase-negative staphylococci (CNS), *Escherichia coli*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, streptococci other than *Strep. dysgalactiae* and *Strep. uberis,* and culture-negative samples. Pathogen-specific CM was determined on lactation level. Pathogen-specific cases of CM were scored as categorical traits, so when in a lactation all cases of CM were caused by one of the above mentioned seven pathogens, a 1 was registered for this particular pathogen, otherwise it was scored as 0. The group of remaining cases of CM consisted of all lactations in which (a) cases of CM were associated with any other pathogen or mixed cultures or (b) consecutive cases of CM were not caused by the same pathogen. In total, 3,393 lactations were recorded with cases of CM before 450 DIM (Table 4.1).

Data selection. Originally, phenotypic records on CM and bacteriological characterisation were available on 49,529 lactations that had been recorded for at least one day during the study. Records of cows with unknown pedigree, or with extreme ages at calving were deleted. This reduced the dataset to 47,563 lactations (De Haas et al., 2002b). For the present study, only lactations of cows that had calved after the starting date of the study on farm were included in the dataset, resulting in a dataset with only lactations that were recorded in the study from calving onwards. This criterion was set to ensure that no previous cases of CM had occurred within the same lactation, and the dataset was reduced to 26,427 lactations. Lactations with fewer than 6 recordings for SCC were deleted from the dataset, to enable sufficient time for a complete pattern to be present. The final dataset consisted of 24,012 lactations from 19,733 cows. Somatic cell count was recorded on 245,595 test-days before 450 DIM.

Definition of patterns. To determine the patterns of peaks in SCC, SCC was expressed relative to SCC in lactations without CM and SCM (De Haas et al., 2002a), by first estimating SCC on each DIM of these lactations with a spline function (Gilmour et al., 2002). Usually, a spline function is used for smoothing data points and the function allows maximum flexibility and assumes no prescribed curvature (Gilmour et al., 2002). Secondly, these estimates were subtracted from the SCC test-day records for each DIM value. The procedure was carried out separately for heifers and multiparous cows, and this calculated value for SCC is referred to as the corrected SCC (CSCC). Because De Haas et al. (2002a) only used SCC up to 308 DIM, SCC was extrapolated from 309 to 450 DIM, by adding the average daily increase in SCC from 100 to 300 DIM to the calculated SCC on the previous day. Here the assumption was made that milk yield showed a linear association with DIM at the end of the lactation (>309 DIM), so SCC could be linearly extrapolated with DIM.

Five SCC patterns were defined and distinctions were made between lactations with short or longer periods of increased CSCC, and also between lactations with and without recovery within three or five test-day recordings. A recovery is classified by a recording of a low CSCC after a rise in CSCC. Pattern 1 describes a quick rise in CSCC followed by a quick decrease in CSCC, and is referred to as “quick recovery pattern”. Three consecutive test-day records of CSCC had to be low, high, and low again (Figure 4.1). Pattern 2, referred to as ”slow recovery pattern”, described a slower increase in CSCC, but still with recovery, i.e. low, higher, high, lower, and low again (Figure 4.1). Pattern 3 had no restrictions on CSCC on the second and fourth test-day record, but the first one had to be low, the third one had to be high, and the fifth one had to be low again. There is overlap between patterns 2 and 3, because of equal restrictions for the first, third and fifth test-day. Pattern 4 captured a longer increased SCC; i.e. low CSCC followed by four high CSCC, so no recovery took place within four test-day recordings. Pattern 4 is referred to as “no recovery pattern” (Figure 4.1). To

compare these patterns with the lactation-average SCC, the fifth trait was a binary parameter that indicated whether the lactation-average SCC was higher or lower than 200,000 cells/ml (scored as 1 or 0, respectively) (SCC200).

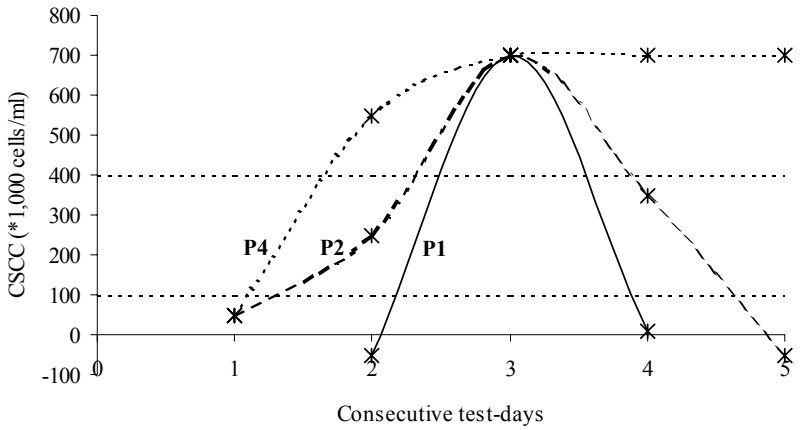

Figure 4.1 *The solid line shows an example of a short, quick increase in somatic cell counts, relative to SCC for lactations without elevated SCC (CSCC); i.e. the "quick recovery pattern" (P1). The broken line shows an example of a slow increase in somatic cell count, but with recovery within five consecutive test-days; i.e. the "slow recovery pattern" (P2). The dotted line shows an example of a long increased somatic cell count, without recovery within five consecutive test-days; i.e. the "no recovery pattern" (P4).*

Upper and lower thresholds for SCC were set based on findings in literature. Healthy and recovered cows were assumed to have less than 200,000 somatic cells/ml (Dohoo and Leslie, 1991; Smith et al., 2001). Infected cows were assumed to produce more than 500,000 cells/ml (Lam et al., 1997). These thresholds for SCC are in accordance with a threshold of 100,000 cells/ml for low CSCC and a threshold of 400,000 cells/ml for high CSCC, since the average CSCC was 100,000 cells/ml. Although CSCC of zero represented the standard curve for lactations without CM and SCM, existing variation around this curve was taken into account when setting the thresholds for CSCC. The choice of thresholds, although objective, was arbitrary. Lower thresholds for CSCC of 50,000 and 150,000 cells/ml, and an upper threshold for CSCC of 650,000 cells/ml were also evaluated.

The first criterion of all patterns of peaks in SCC is a low CSCC. However, to be able to identify cows with high CSCC directly after calving, the criteria for the definitions were set differently in the beginning of the lactation. At the end of the lactation, the criteria for the definitions were set differently as well, because cows might have been culled before they were recorded on the required number of test-days included in the definition of the patterns of peaks in SCC. Therefore, in the beginning of the lactation, only the second half of the patterns of peaks in SCC was used, and at the end of the lactation, the first half of the patterns of peaks in SCC was used. With three consecutive missing recordings on SCC, it was assumed that the cow was either culled, or dried off.

Patterns of peaks in SCC were scored as categorical traits, so when in a lactation a pattern was present in three or five consecutive test-day recordings, it was registered as 1, and otherwise as 0. More than one pattern could be present in one lactation (Table 4.1).

Odds ratio, sensitivity, specificity, and predictive values. Odds ratios (OR), sensitivities and specificities were used to describe the associations between presence of patterns and incidence of pathogen-specific CM in lactations. Odds ratio are measures of associations, and indicate the change in risk. The sensitivity is the probability that a diseased animal (here defined as a lactation with occurrence of a pathogen-specific case of CM) indeed will be classified as diseased using the test (here defined as a lactation with presence of any of the defined patterns), and the specificity is the probability that a non-diseased animal will be classified as non-diseased with the test (Noordhuizen et al., 1997). Specificity in this study is a relative specificity, since our golden standard is not without error because of variation between farmers in diagnostic criteria (Noordhuizen et al., 1997). The positive predictive value of a test is the probability that in a lactation with a given SCC pattern a case of CM with a given pathogen is diagnosed by the farmer. The negative predictive value is the probability that a case of CM with a given pathogen is not diagnosed in a lactation without a given SCC pattern. Contingency tables are fully filled, indicating that there are also lactations scored with a case of CM, but without presence of any of the patterns, and vice versa, i.e. lactations without a case of CM, but with presence of a pattern.

The probabilities for occurrence of pathogen-specific CM in lactations with presence of a SCC pattern were calculated with single-trait regressions, and so were the probabilities for presence of a SCC pattern in lactations with occurrence of pathogen-specific CM. Both single-trait regressions were applied on two datasets; one including all available lactations (n = 24,012) (= dataset 1), and one including only the lactations with CM (n = 3,393) (= dataset 2). These datasets distinguish between probabilities to classify a diseased cow out of all cows (dataset 1), and to identify the involved pathogen in cows with CM (dataset 2).

The effect of occurrence of pathogen-specific CM on the presence of one of the patterns was also investigated with a multi-variate regression. Only one pathogen can occur in a lactation, and therefore pathogen-specific cases of CM are fully independent. Odds ratios, therefore, indicate probabilities on occurrence of specific pathogens versus no CM (dataset 1), or versus the group of remaining records (dataset 2).

Multi-variate regression was also applied to investigate the effect of presence of any of the patterns in a lactation on occurrence of pathogen-specific CM. More than one pattern can be present in a lactation, so patterns are not independent. Odds ratios indicate the probability of presence of a certain pattern versus no presence of patterns 1, 2, 3 or 4 or SCC200, independent of the analysed dataset. Fit of the models was evaluated based on the estimated deviance of the model.

Table 4.1 *Total number of lactations with no clinical mastitis (CM) and with pathogen-specific CM, number of lactations where consecutive test-day records for SCC showed one of the newly defined patterns in SCC[1], and number of lactations with lactation-average SCC of more than 200,000 cells/ml (SCC200)*

	No.	P1	P2	P3	P4	SCC200
No clinical mastitis	20,619	1,950	246	2,157	443	4,758
Staphylococcus aureus	394	57	18	84	52	291
Coagulase-negative staphylococci	117	14	5	20	13	50
Escherichia coli	624	156	17	157	25	264
Streptococcus dysgalactiae	220	51	7	58	10	137
Streptococcus uberis	159	35	6	39	15	95
Other streptococci	185	45	3	51	17	98
Culture-negative samples	493	88	9	99	18	174
Other pathogens	1,201	265	29	291	118	728
Total	24,012	2,661	340	2,956	711	6,595

[1] P1 (pattern 1): low-high-low SCC; P2 (pattern 2): low-higher-high-lower-low SCC; P3 (pattern 3): low-no restrictions-high-no restrictions-low SCC; P4 (pattern 4): low-high-high-high-high SCC.

Statistical analyses. Statistical analyses were carried out to test if ORs differed from 1, using logistic regression in SAS (PROC GENMOD; (SAS/STAT®, 2001)). For investigating the effect of occurrence of pathogen-specific CM on presence of patterns, the

analyses were done separately for each pattern (1, 2, 3 and 4), and for SCC200, with this general model:

$$\text{Logit}(\text{SCC pattern}_j) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_k X_k + e_{jk} \quad [1]$$

where the outcome variable is a binary indicator variable (0/1) for any of the patterns, with j = 0…5 (pattern 1 – 4, or SCC200), α = intercept and e = residual random error. The modelled probability was presence of a pattern, and included regression coefficients were pathogen-specific CM ($\beta_{1\text{-}8}$), parity (β_9) and the fraction of HF genes (β_{10}). Eight classes for pathogen-specific CM were defined, i.e. seven classes for defined pathogens, and one group of remaining records. Pathogen-specific CM was recorded as a binary trait (0/1).

For investigating the effect of presence of patterns on occurrence of pathogen-specific CM, the analyses were done separately for each pathogen, with this general model:

$$\text{Logit}(\text{CM}_j) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_k X_k + e_{jk} \quad [2]$$

where the outcome variable is a binary indicator variable (0/1) for pathogen-specific cases of CM, with j = 0…8, α = intercept and e = residual random error. The modelled probability was occurrence of pathogen-specific CM, and regression coefficients were estimated for the SCC patterns ($\beta_{1\text{-}4}$), SCC200 (β_5), parity (β_6) and the fraction of HF genes (β_7).

Parity had 2 classes, distinguishing between heifers and multiparous cows, and 5 classes of fractions of HF genes were defined (0, 1/4, …, 4/4). Herd was included as a repeated variable (274 classes).

Results

Effect of parity and Holstein% on patterns. When analysing all lactations, incidence of patterns of peaks in SCC was lower in heifers than in multiparous cows ($p < 0.05$). When analysing only the lactations with CM, the "no recovery pattern" was significantly ($p < 0.05$) less present in heifers compared to multiparous cows. No significant associations were estimated for the other patterns.

Long increased SCC was more frequent in cows with 26-75% HF, compared to cows with 76-100% HF. Furthermore, more lactations of cows with ≤75% HF had averages of SCC of >200,000 cells/ml, than of cows with 76-100% HF. Similar results were found in dataset 2; i.e. lactations of cows with 26-50% HF showed more often high averages of SCC (>200,000 cells/ml), than lactations of cows with 76-100% HF.

Table 4.2 *Calculated odds ratio (OR) for the effect of patterns of peaks in somatic cell count (SCC)[1] and lactation-average SCC of more than 200,000 cells/ml (SCC200) on pathogen-specific clinical mastitis (CM). Sensitivity (SE) and specificity (SP) indicating that lactations with or without pathogen-specific CM are or are not detected by a certain pattern. Predictive values, positive and negative (PV+, PV-), to indicate the probability that a SCC pattern correctly predicts presence or absence of pathogen-specific CM. Analyses are performed for all available lactations and for only lactations with CM, separately*

	All lactations					Only lactations with CM				
	OR	SE	SP	PV+	PV-	OR	SE	SP	PV+	PV-
Staphylococcus aureus										
Pattern 1	1.36	0.14	0.89	0.02	0.98	0.61	0.14	0.78	0.08	0.87
Pattern 2	3.46	0.05	0.99	0.05	0.98	1.84	0.05	0.97	0.19	0.89
Pattern 3	1.96	0.21	0.88	0.03	0.99	0.87	0.21	0.76	0.11	0.88
Pattern 4	5.29	0.13	0.97	0.07	0.99	1.96	0.13	0.93	0.19	0.89
SCC200	7.76	0.74	0.73	0.04	0.99	2.66	0.74	0.48	0.16	0.93
Coagulase neg. staphylococci										
Pattern 1	1.09	0.12	0.89	0.01	0.99	0.50	0.12	0.79	0.02	0.96
Pattern 2	3.14	0.04	0.99	0.01	0.99	1.60	0.04	0.97	0.05	0.97
Pattern 3	1.47	0.17	0.88	0.01	0.99	0.66	0.17	0.76	0.03	0.96
Pattern 4	4.15	0.11	0.97	0.02	0.99	1.48	0.11	0.92	0.05	0.97
SCC200	1.98	0.43	0.73	0.01	0.99	0.62	0.43	0.45	0.03	0.96
Escherichia coli										
Pattern 1	2.78	0.25	0.89	0.06	0.98	1.33	0.25	0.80	0.22	0.83
Pattern 2	2.00	0.03	0.99	0.05	0.97	0.98	0.03	0.97	0.18	0.82
Pattern 3	2.47	0.25	0.88	0.05	0.98	1.11	0.25	0.77	0.23	0.82
Pattern 4	1.38	0.04	0.97	0.04	0.97	0.43	0.04	0.91	0.09	0.81
SCC200	1.98	0.42	0.73	0.04	0.98	0.56	0.42	0.43	0.14	0.77
Streptococcus dysgalactiae										
Pattern 1	2.45	0.23	0.89	0.02	0.99	1.15	0.23	0.79	0.07	0.94
Pattern 2	2.32	0.03	0.99	0.02	0.99	1.17	0.03	0.97	0.07	0.94
Pattern 3	2.58	0.26	0.88	0.02	0.99	1.18	0.26	0.77	0.07	0.94
Pattern 4	1.57	0.05	0.97	0.01	0.99	0.53	0.05	0.92	0.04	0.93
SCC200	4.43	0.62	0.73	0.02	0.99	1.43	0.62	0.46	0.07	0.95

Continued overleaf

	All lactations					Only lactations with CM				
	OR	SE	SP	PV+	PV-	OR	SE	SP	PV+	PV-
Streptococcus uberis										
Pattern 1	2.28	0.22	0.89	0.01	0.99	1.07	0.22	0.79	0.05	0.95
Pattern 2	2.76	0.04	0.99	0.02	0.99	1.40	0.04	0.97	0.06	0.95
Pattern 3	2.33	0.25	0.88	0.01	0.99	1.06	0.25	0.76	0.05	0.95
Pattern 4	3.47	0.09	0.97	0.02	0.99	1.23	0.09	0.92	0.06	0.95
SCC200	3.96	0.59	0.73	0.01	0.99	1.27	0.59	0.46	0.05	0.96
Other streptococci										
Pattern 1	2.61	0.24	0.89	0.02	0.99	1.23	0.24	0.79	0.06	0.95
Pattern 2	1.15	0.02	0.99	0.01	0.99	0.56	0.02	0.97	0.03	0.94
Pattern 3	2.74	0.28	0.88	0.02	0.99	1.25	0.28	0.77	0.06	0.95
Pattern 4	3.37	0.04	0.97	0.02	0.99	1.19	0.04	0.92	0.06	0.95
SCC200	3.00	0.53	0.73	0.01	0.99	0.95	0.53	0.46	0.05	0.94
Culture-negative samples										
Pattern 1	1.77	0.18	0.89	0.03	0.98	0.79	0.18	0.79	0.12	0.85
Pattern 2	1.30	0.02	0.99	0.03	0.98	0.61	0.02	0.97	0.10	0.85
Pattern 3	1.82	0.20	0.88	0.03	0.98	0.79	0.20	0.76	0.12	0.85
Pattern 4	1.25	0.04	0.97	0.03	0.98	0.40	0.04	0.91	0.07	0.85
SCC200	1.45	0.35	0.73	0.03	0.98	0.41	0.35	0.43	0.09	0.79
Other pathogens										
Pattern 1	2.41	0.22	0.89	0.10	0.96	1.11	0.22	0.80	0.37	0.65
Pattern 2	1.79	0.02	0.99	0.09	0.95	0.81	0.02	0.97	0.31	0.64
Pattern 3	2.42	0.24	0.88	0.10	0.96	1.06	0.24	0.77	0.36	0.65
Pattern 4	4.08	0.10	0.97	0.17	0.95	1.48	0.10	0.93	0.44	0.65
SCC200	4.44	0.61	0.74	0.11	0.97	1.50	0.61	0.49	0.40	0.69

[1] Pattern 1: low-high-low SCC; pattern 2: low-higher-high-lower-low SCC; pattern 3: low-no restrictions-high-no restrictions-low SCC; pattern 4: low-high-high-high-high SCC.

Single-trait analyses for associations between pattern and pathogen. All calculated ORs in dataset 1 are above 1, indicating that a diseased cow can be classified out of all cows by presence of SCC patterns (Table 4.2). Odds ratios for SCC200 were highest for cases of CM associated with *Staph. aureus*, *Strep. dysgalactiae*, *Strep. uberis* and the group of remaining records. Cases of CM associated with CNS or streptococci other than *Strep. dysgalactiae* and *Strep. uberis* showed highest ORs for the "no recovery pattern". Chances on presence of the "quick recovery pattern" were high in lactations with clinical *E. coli* mastitis compared to presence of another SCC pattern. No single unambiguous SCC pattern was present for culture-negative samples.

For all pathogens, sensitivities for SCC200 were higher than the sensitivities for any pattern of peaks in SCC (Table 4.2). Pattern 3 was always more sensitive than the other patterns, independent of the pathogen, and the "slow recovery pattern" showed lowest sensitivity for all pathogens, when analysing dataset 1. Specificities were always lowest for SCC200, and highest for the "slow recovery pattern". However, despite the high specificity of the "slow recovery pattern" this is probably not the best trait to detect lactations with pathogen-specific CM because of the low sensitivity.

The negative predictive values were high, especially when analysing all lactations, compared to analysing only lactations with CM (Table 4.2). This indicated that in at least 95% of all lactations, absence of pathogen-specific CM was correctly predicted by absence of any of the defined patterns of peaks in SCC. However, probabilities that presence of a pattern correctly predicted occurrence of pathogen-specific CM were low (≤17%), when analysing dataset 1. When analysing dataset 2, positive predictive values were higher compared with dataset 1. In lactations with CM and with presence of the "no recovery pattern" the predictive value for *Staph. aureus* is 19%, whereas this is only 9% for *E. coli*. On the other hand, the "quick recovery pattern" predicted 22% of the cases of *E. coli* mastitis in lactations with CM, and only 8% of the cases of *Staph. aureus* mastitis.

Multi-variate analyses for effect of pathogens on pattern. Slightly higher ORs were calculated with multi-variate analyses (Table 4.3 and Table 4.4), compared with single-trait analyses (Table 4.2), especially when analysing dataset 1.

Clinical *E. coli* mastitis was significantly associated with an increased risk on presence of a "quick recovery pattern" (OR = 3.14) (Table 4.3). Clinical *Staph. aureus* or CNS mastitis was associated with increased risk on presence of a "no recovery pattern", with ORs of above 6. Clinical *Staph. aureus* or CNS mastitis was also associated with increased odds of a "slow recovery pattern", compared to lactations with presence of a "slow recovery pattern", but without a case of CM (OR = 3.71 and 3.75, respectively).

In lactations with CM, the odds of "quick recovery pattern" (versus none of the SCC patterns being present) were roughly 50% when *Staph. aureus* or CNS were isolated (OR = 0.60 and 0.47, respectively) (Table 4.4). Probabilities of observing cases of CM associated with *E. coli*, *Strep. dysgalactiae* or culture-negative samples were low, compared to lactations with presence of a "no recovery pattern" and a case of CM associated with the group of remaining records.

High ORs were estimated for SCC200 when analysing dataset 1 (Table 4.3), indicating higher chances of finding high lactation-average SCC in lactations with pathogen-specific CM than in lactations without CM. Among the lactations with CM, *Staph. aureus* was the only pathogen which was associated with an increased odds of SCC200, compared to the group of remaining records (Table 4.4). Most other pathogens were associated with a decreased odds of SCC200, compared to the group of remaining records, although *Strep. dysgalactiae* and *Strep. uberis* differed not significantly from 1.

Changing the threshold values had no severe impact on the associations between occurrence of pathogen-specific CM and presence of patterns. Interpretations of ORs were similar, independent of the threshold values.

Table 4.3 *The odds ratio for the effect of pathogen-specific clinical mastitis (CM) on four patterns of peaks in somatic cell count (SCC)*[1] *and lactation-average SCC of more than 200,000 cells/ml (SCC200), separately, when analysing all available lactations*

	Pattern 1	Pattern 2	Pattern 3	Pattern 4	SCC200
Staphylococcus aureus	1.63 *	3.71 *	2.31 *	6.24 *	9.31 *
Coagulase-negative staphylococci	1.34	3.75 *	1.82 *	6.31 *	2.94 *
Escherichia coli	3.14 *	2.14 *	2.79 *	1.74 *	2.33 *
Streptococcus dysgalactiae	2.90 *	2.62 *	3.06 *	2.04 *	5.41 *
Streptococcus uberis	2.67 *	3.08 *	2.70 *	4.09 *	4.86 *
Other streptococci	3.02 *	1.29	3.17 *	4.19 *	3.68 *
Culture-negative samples	2.15 *	1.44	2.19 *	1.72 *	2.00 *
Other pathogens	2.73 *	1.92 *	2.70 *	4.49 *	4.97 *

[1] Pattern 1: low-high-low SCC; pattern 2: low-higher-high-lower-low SCC; pattern 3: low-no restrictions-high-no restrictions-low SCC; pattern 4: low-high-high-high-high SCC.

* Significantly different from 1, with $p < 0.05$.

Table 4.4 *The odds ratio for the effect of pathogen-specific clinical mastitis (CM) on four patterns of peaks in somatic cell count (SCC)*[1] *and lactation-average SCC of more than 200,000 cells/ml (SCC200), separately, when analysing only lactations with CM*

	Pattern 1	Pattern 2	Pattern 3	Pattern 4	SCC200
Staphylococcus aureus	0.60 *	1.91 *	0.85	1.39	1.94 *
Coagulase-negative staphylococci	0.47 *	1.80	0.64	1.27	0.56 *
Escherichia coli	1.18	1.14	1.05	0.37 *	0.47 *
Streptococcus dysgalactiae	1.06	1.33	1.12	0.45 *	1.06
Streptococcus uberis	0.98	1.58	1.00	0.96	0.96
Other streptococci	1.13	0.67	1.18	0.95	0.75 *
Culture-negative samples	0.77	0.74	0.79	0.38 *	0.39 *

[1] Pattern 1: low-high-low SCC; pattern 2: low-higher-high-lower-low SCC; pattern 3: low-no restrictions-high-no restrictions-low SCC; pattern 4: low-high-high-high-high SCC.
* Significantly different from 1, with $p < 0.05$.

Multi-variate analyses for effect of patterns on pathogen. With multi-variate analyses, lower ORs were calculated (Table 4.5), compared with single-trait analyses (Table 4.2), especially when analysing dataset 1. Different combinations of patterns included in the model resulted in different estimates for OR, and possibilities to elucidate specific mastitis-causing pathogens differed as well. For *Staph. aureus*, a better fit was found for the model including all patterns versus separate models with each pattern. This indicated that for *Staph. aureus* a model can be defined including a combination of the patterns, resulting in an optimal fit of the model (results not shown). For other pathogens, including more patterns in the model did not improve the fit of the model, so a model with only one pattern is preferred (results not shown).

All patterns except the "slow recovery pattern" were significantly associated with increased risk on overall CM, whereas the "slow recovery pattern" was associated with a decreased risk on overall CM (Table 4.5). The "no recovery pattern" and SCC200 were associated with increased odds of clinical *Staph. aureus* mastitis, but the "quick recovery pattern" was associated with decreased odds of *Staph. aureus*. This implied that presence of a long increased SCC, without recovery within four consecutive test-days, was rather common in lactations with clinical *Staph. aureus* mastitis, as well as presence of a high lactation-average SCC, but presence of the "quick recovery pattern" was not so common. When analysing dataset 1, SCC200 was associated with an increased risk on clinical CNS mastitis, whereas, when analysing only lactations with CM (dataset 2), SCC200 was associated with a decreased risk on CNS. This implied a higher risk on occurrence of CNS in lactations with

high averaged SCC. Among lactations with CM, CNS mastitis did not occur more often in lactations with high lactation-average SCC. The "quick recovery pattern" was significantly more often present in lactations with clinical *E. coli* mastitis, as compared to peaks in SCC not described by pattern 1, 2, 3 or 4 or SCC200. For cases of CM associated with one of the streptococci or a culture-negative sample no clear associations with a SCC pattern in particular were found.

Discussion

Effect of parity and Holstein% on presence of patterns. Lactation-average SCC increases with increasing parity (Blackburn, 1966; Lindström et al., 1981). In the current study, SCC adjusted for SCC of healthy cows (CSCC) was used, which was also corrected for lactation stage and parity effect. Still, the number of peaks in CSCC increased with increasing parity. This was especially true for the "no recovery pattern", where 61% of all lactations with the presence of this pattern were from fourth or higher parity cows, whereas 35, 44 and 36% of all lactations with the presence of patterns 1, 2 and 3, respectively, were from fourth or higher parity cows. The number of cases of CM also increased with increasing parities (Detilleux et al., 1995; Martin et al., 2002). In the current study, CM was recorded in 9.3, 13.3, 16.3, and 18.6% of all lactations from cows in parities 1, 2, 3 and ≥4, respectively. This might explain the higher number of peaks for older cows, compared with cows of first, second or third parity.

Patterns of peaks in SCC are more often present in cows with relatively low percentages of HF. This might be (partly) explained by the higher incidences of cases of CM in cows with ≤75% HF, compared to cows with >76% HF. This distribution has been presented before by De Haas et al. (2002a).

Biological interpretation of patterns. Intramammary infections with *Staph. aureus* are often characterised by a long duration and high SCC (Sears et al., 1990; Daley et al., 1991). Although, a wave-like pattern of increased SCC may be present (Daley et al., 1991). Cases of clinical *Staph. aureus* mastitis occurred more frequently in later lactation (Vaarst and Enevoldsen, 1997), and may therefore be more easily detected using the patterns. As expected, *Staph. aureus* was more often associated with the "no recovery pattern", and less often with the "quick recovery pattern".

Table 4.5 *The odds ratio (OR) and the 95% confidence interval for the effect of patterns of peaks in somatic cell count (SCC)[1] and lactation-average SCC of more than 200,000 cells/ml (SCC200) on pathogen-specific clinical mastitis (CM), when analysing all available lactations or only the lactations with CM, separately*

	All lactations			Only lactations with CM		
	OR[2]	95% Confidence interval		OR[2]	95% Confidence interval	
Overall clinical mastitis						
Pattern 1	1.37	1.20	1.56			
Pattern 2	0.74	0.56	0.97			
Pattern 3	1.47	1.29	1.68			
Pattern 4	1.91	1.63	2.23			
SCC200	3.17	2.89	3.48			
Staphylococcus aureus						
Pattern 1	0.70	0.51	0.95	0.57	0.42	0.77
Pattern 2	1.10	0.63	1.94	1.58	0.88	2.82
Pattern 3	1.12	0.81	1.54	0.85	0.62	1.16
Pattern 4	1.77	1.28	2.45	1.14	0.80	1.62
SCC200	7.44	5.68	9.73	2.96	2.25	3.91
Coagulase-negative staphylococci						
Pattern 1	0.83	0.47	1.44	0.62	0.36	1.06
Pattern 2	1.38	0.47	4.04	1.85	0.65	5.28
Pattern 3	1.20	0.61	2.36	0.84	0.45	1.55
Pattern 4	2.89	1.51	5.57	1.88	0.99	3.56
SCC200	1.79	1.15	2.82	0.65	0.43	1.00
Escherichia coli						
Pattern 1	1.96	1.54	2.49	1.48	1.17	1.86
Pattern 2	1.16	0.64	2.10	1.34	0.74	2.42
Pattern 3	1.35	1.02	1.77	0.97	0.75	1.27
Pattern 4	1.00	0.64	1.55	0.58	0.38	0.89
SCC200	1.45	1.20	1.74	0.53	0.44	0.64

Continued overleaf

	All lactations			Only lactations with CM		
	OR[2]	95% Confidence interval		OR[2]	95% Confidence interval	
Streptococcus dysgalactiae						
Pattern 1	1.23	0.78	1.94	1.00	0.67	1.49
Pattern 2	1.00	0.45	2.21	1.07	0.48	2.39
Pattern 3	1.35	0.88	2.06	1.02	0.69	1.50
Pattern 4	0.72	0.38	1.38	0.46	0.24	0.87
SCC200	4.14	3.01	5.69	1.56	1.17	2.07
Streptococcus uberis						
Pattern 1	1.34	0.80	2.23	1.05	0.65	1.68
Pattern 2	1.09	0.42	2.84	1.36	0.56	3.30
Pattern 3	1.25	0.74	2.09	0.94	0.59	1.51
Pattern 4	1.63	0.89	2.96	1.08	0.61	1.93
SCC200	3.16	2.16	4.62	1.22	0.84	1.77
Other streptococci						
Pattern 1	1.34	0.84	2.13	1.05	0.68	1.60
Pattern 2	0.33	0.09	1.15	0.40	0.12	1.42
Pattern 3	1.86	1.24	2.79	1.38	0.95	1.99
Pattern 4	2.26	1.25	4.08	1.42	0.78	2.57
SCC200	2.12	1.53	2.94	0.86	0.63	1.16
Culture-negative samples						
Pattern 1	1.31	0.99	1.73	0.94	0.71	1.23
Pattern 2	0.80	0.40	1.61	0.85	0.40	1.79
Pattern 3	1.44	1.06	1.96	0.98	0.72	1.34
Pattern 4	1.08	0.67	1.72	0.67	0.41	1.10
SCC200	1.32	1.05	1.65	0.46	0.37	0.58

[1] Pattern 1: low-high-low SCC; pattern 2: low-higher-high-lower-low SCC; pattern 3: low-no restrictions-high-no restrictions-low SCC; pattern 4: low-high-high-high-high SCC.

[2] Odds ratio for having a lactation with the occurrence of a certain pattern, compared to lactations with the occurrence of any other pattern, not described by pattern 1, 2, 3 or 4 or SCC200.

Phenotypic associations in the current study showed that clinical *E. coli* mastitis is associated with short peaks in SCC. *Escherichia coli* infections are typically acute cases (Vaarst and Enevoldsen, 1997), which agrees with the strong association found between the "quick recovery pattern" SCC and clinical *E. coli* mastitis in the current study. These results are confirmed by studies that used either induced cases, or naturally occurring cases of CM. The effect of naturally occurring cases of clinical *E. coli* mastitis on the curve of SCC during lactation has been described using the same dataset (De Haas et al., 2002a). Before a case of clinical *E. coli* mastitis, SCC was close to the SCC level of lactations without CM and SCM, and after the case SCC came down to a level that was only slightly higher than the preinfection level. From experiments inducing *E. coli*, it was shown that SCC peaks two days after inoculation, and preinfection SCC values returned within three to four weeks after challenge (Lohuis et al., 1990; Erskine et al., 1992; Kremer et al., 1993; Pyörälä et al., 1994). In contrast, within 24 h after inoculation with *Staph. aureus*, SCC increased and remained high for at least 48 days (Shoshani et al., 2000). Culture-negative CM showed strong similarities with clinical coliform mastitis (Vaarst and Enevoldsen, 1997), and associations between patterns and culture-negative CM in the current study were indeed similar to those with *E. coli* mastitis.

Based on the results in the current study, clinical *Strep. dysgalactiae* mastitis was not strongly associated with the presence of one pattern in particular, because ORs were around 1, which agrees with the specific characteristics of *Strep. dysgalactiae* reported by Vaarst and Enevoldsen (1997). Leigh (1999) concluded that clinical *Strep. uberis* mastitis can be associated with longer increased SCC, which was also established in the current study when analysing dataset 1.

While interpreting the patterns of peaks in SCC, it should be taken into account that the pattern after the rise in SCC is affected by the type of therapy of the cow. However, since all cows with CM were treated, this effect is present for each pattern. In practice cows are treated as well, hence the data used in this study correspond with data that is available to farmers. Responses to the therapy might differ per cow, and with the data analysed in this study it is impossible to specify whether cure occurs spontaneous or as a result of therapy, but that is also unknown in practical situations. To diagnose individual cows by using the SCC patterns might not be the best method, because of the time needed to complete a pattern. For individual cases bacteriology of milk samples will provide quicker and more accurate results. However, when analysing these SCC patterns at herd level, a predominant pathogen picture might occur. This can help with introducing pathogen-specific mastitis control programs, consisting of adequate guidelines to control intramammary infections with both contagious and environmental pathogens.

Comparison of pattern 1 – 4 with SCC200. Although it is not exactly the same trait as the lactation-average SCC, SCC200 was defined to be able to make the comparison with average lactation values of SCC, which is currently used to monitor udder health. The major difference is that lactation-average SCC gives as much weight to a reduction of 150,000 to 50,000 cells/ml, as from 900,000 to 800,000 cells/ml, whereas SCC200 only distinguishes between high versus low lactation-average SCC. Therefore, associations between lactation-average SCC and pathogen-specific CM might differ from those found for SCC200. Heuven (1987) compared several SCC variables as predictors of bacteriological status, and concluded that cows with high deviations from the typical curve of SCC during lactation were more likely to be treated for CM. Still, lactation-average SCC was a more accurate predictor of pathogens than a single SCC measure on bacteriological sample day (Heuven, 1987). Based on comparison of the positive predictive values, occurrence of pathogen-specific CM was equally or even more correctly predicted by presence of one of the SCC patterns, than by presence of SCC200. Lactations with presence of the "no recovery pattern" had more chance on occurrence of clinical *Staph. aureus* mastitis, compared with the base probability of *Staph. aureus* (2%) (Table 4.1). Higher probabilities than the base probability were found for *E. coli* when the "quick recovery pattern" was present in a lactation. Lactations with CM and with presence of the "no recovery pattern" had high probabilities on clinical *Staph. aureus* or CNS mastitis, but low probabilities on *E. coli* or culture-negative CM. The opposite was true for lactations with presence of the "quick recovery pattern". The results support the hypothesis that a different definition of SCC can increase the predictive value of the bacteriological status of a cow, but a combination of several definitions of SCC might be necessary to capture the full scope of mastitis-causing pathogens. Patterns of peaks in SCC may be useful as basic tools for health management advises, as they do provide additional information on, for example, the incidence of (spontaneous or therapeutic) cure, in comparison with the information provided by lactation-average SCC alone. Further analyses will be done on the distributions of the SCC patterns on farms, and whether this is informative for pathogen-distribution on that farm. Genetic analyses will be carried out as well to establish if decreased genetic susceptibility to mastitis-causing pathogens can be achieved by genetic selection on patterns of peaks in SCC.

Further optimisation of trait definition. Several improvements in the current analyses can be made, which are expected to increase the detection of the SCC patterns, or to increase the chance of finding an association between SCC patterns and CM. For example, noise in SCC test-day recordings might be removed from the data by correcting for fixed effects, like herd-test-date, in the statistical model. Chances of finding a peak in SCC were also determined by the frequency of the test-day recordings. Recording an increased SCC on the test-day depended on both the day of occurrence of cases of CM in relation to test-day recordings and the duration of increased SCC as a result of pathogen-specific CM. Using this

additional information will mainly help to detect acute cases of CM, but not the chronic cases since SCC remains high for some time with a chronic case of CM. Therefore chances of detecting such a high SCC are higher as compared to detect an increased SCC as a result of an acute case of CM, since SCC rises quickly and is low before and after an acute case of CM.

Conclusions

The presence of patterns in a lactation is informative for the pathogen-specific incidence of CM. Patterns can distinguish between cases of pathogen-specific CM, and they predict the occurrence of pathogen-specific CM equally or even more accurately than SCC200. Lactations with CM and with presence of the "no recovery pattern" had high probabilities for clinical *Staph. aureus* or CNS mastitis, but low probabilities for *E. coli* or culture-negative CM. The opposite was true for lactations with presence of the "quick recovery pattern". Presence of patterns of peaks in SCC can be partly explained by pathogen-specific mammary gland pathogenesis, since pathogens may have typical characteristics.

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Chapter 5

Genetic associations for pathogen-specific clinical mastitis and patterns of peaks in somatic cell count

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Abstract

Genetic associations were estimated between pathogen-specific cases of clinical mastitis (CM), lactation-average somatic cell score (LACSCS), and patterns of peaks in somatic cell count (SCC) which were based on deviations from the typical lactation curve for SCC. The dataset contained test-day records on SCC in 94,781 lactations of 25,416 cows of different parities. Out of these 94,781 lactations, 41,828 lactations had recordings on occurrence of pathogen-specific CM and on SCC, and 52,953 lactations had recordings on SCC only. A total of 5,324 lactations with cases of CM were recorded. Analysed pathogens were *Staphylococcus aureus*, coagulase-negative staphylococci, *Escherichia coli*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, and culture-negative samples. Pattern definitions were based on three or five consecutive test-day recordings of SCC. They differentiated between short or longer periods of increased SCC, and also between lactations with and without recovery. Occurrence of pathogen-specific CM and presence of patterns of peaks in SCC were both scored as binary traits. Variance components for sire, maternal grandsire, and permanent animal effects were estimated using AS-REML. The estimated heritability for overall CM was 0.04, and similar heritabilities for pathogen-specific CM were estimated. Heritabilities for the patterns of peaks in SCC ranged from 0.01 to 0.06. Heritabilities for LACSCS were 0.07 to 0.08. Genetic correlations with patterns of peaks in SCC differed for each pathogen. Generally, genetic correlations between pathogen-specific CM and patterns of peaks in SCC were stronger than the correlations with LACSCS. This suggests that genetic selection purely on diminishing presence of peaks in SCC would decrease the incidence of pathogen-specific CM more effectively than selecting purely on lower LACSCS.

Keywords Genetic correlation, Clinical mastitis, Pathogens, Somatic cell count

Introduction

Current selection indices realise an increase in milk yield and simultaneously monitor udder health by selecting for lower lactation-average somatic cell score (LACSCS). Selection for lower LACSCS is expected to be effective in reducing the incidence of clinical *Escherichia coli* mastitis, but less effective in reducing incidences of clinical *Staphylococcus aureus* and *Streptococcus dysgalactiae* mastitis (Nash et al., 2000; De Haas et al., 2002b). Instead of using LACSCS, somatic cell count (SCC) from individual test-day records could be used (Reents et al., 1995). Analysing herd-test-days allows distinguishing between, for example, cows with just one high peak in SCC, and cows with chronically high SCC, although these cows might have the same LACSCS. It could also be hypothesised that patterns of peaks in SCC, which are based on deviations from the typical lactation curve, are more effective selection criteria against (some forms of) clinical mastitis (CM) than LACSCS (Sheldrake et al., 1983; Schepers et al., 1997; De Haas et al., 2002a). Firstly, because different pathological backgrounds of CM result in distinguishable SCC patterns, which cannot be taken into account by LACSCS (Sears et al., 1990; Daley et al., 1991; Vaarst and Enevoldsen, 1997). Secondly, our phenotypic study of patterns of peaks in SCC and pathogen-specific CM did show that the presence of patterns of peaks in SCC in a lactation was informative for the occurrence of pathogen-specific CM (De Haas et al., 2003). For instance, clinical *E. coli* mastitis was associated with the presence of a short peak in SCC, whereas *Staph. aureus* was associated with long increased SCC. The objective of this study was to estimate genetic parameters for pathogen-specific CM, LACSCS, and patterns of peaks in SCC. This was done to establish if these SCC patterns provide additional information for selection that aims to decrease genetic susceptibility to (pathogen-specific) CM, in comparison to the information provided by LACSCS alone.

Material and Methods

Herds. Records on CM were available from December 1992 till June 1994 on 274 Dutch farms (Barkema et al., 1998). The actual start and end date of the study varied slightly among farms, but all farms participated in the study for 18 months. Lactating cows were housed in free-stall barns and milking parlours were double herringbone or two-sided open tandem. Herds participated in a milk recording system, and annual milk production quotas were between 300,000 and 900,000 kg. The national milk recording system (Royal Dutch Cattle Syndicate (NRS), Arnhem, The Netherlands) provided information on three- or four-weekly test-day recordings from 1990 till 1999, from all cows that participated in the longitudinal prospective cohort study of Barkema et al. (1998). A record included national cow identification, breed, date of milk recording, date of calving, date of drying off, test-day milk yields (kilograms milk, fat and protein) and SCC (cells per ml). The breed of the

cow was divided into three subclasses. The main breeds were Holstein-Friesian, Dutch-Friesian and Meuse-Rhine-Yssel.

Bacteriological sampling. Selection of herds and aseptic sampling procedures have been described previously (Barkema et al., 1998). Data collection of milk samples of quarters with CM depended heavily on the willingness of farmers. Therefore, farmers were continuously encouraged, as described by Barkema et al. (1998). During the study period, farmers took milk samples from only those quarters that, in their opinion, had clinical signs of mastitis. Samples were stored in a freezer at the farm (at approximately -20°C) and were collected for bacteriological examination at intervals of six to eight weeks. Bacteriological culturing of milk samples was performed according to the standards of the National Mastitis Council (Harmon et al., 1990). Briefly, 0.01 ml was cultured and for each culture the number of colony-forming units of each of the bacterial species was counted. Collected data contained information on national cow identification number, date of CM occurrence, quarter location, and result of bacteriological culturing. Pathogen-specific CM was coded as a categorical trait (1 = presence, 0 = absence), irrespective of whether the cow had one or more than one case of CM during the lactation. Therefore, more than one pathogen could be present in a lactation. When separate cases of CM associated with different pathogens occurred in a lactation, or when one case with a mixed culture was isolated, the lactation was scored as 1 for several pathogens. More than one case of CM occurred in 20% of all lactations with CM.

Six groups of pathogens were defined based on their incidence in the data: *Staphylococcus aureus*, coagulase-negative staphylococci (CNS), *Escherichia coli*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*. In 19.2% of all bacteriological examinations no pathogen could be isolated (culture-negative), which made the sixth group. A group of remaining records was put together, consisting of lactations with cases of CM caused by other pathogens. In total, 5,324 lactations were recorded with cases of CM occurring before 450 days in milk (DIM). This boundary was established using a histogram, and sufficient data was available per DIM until 450 days.

Data selection. Originally, phenotypic records on CM and bacteriological characterisation were available on 49,529 lactations that had been monitored for at least one day during the study. The data was cleaned up by deleting records of cows with unknown pedigree or with extreme ages at calving (De Haas et al., 2002b). This reduced the dataset to 47,563 lactations from 28,695 cows of different parities. Somatic cell count was recorded between January 1990 and December 1999 in 109,335 lactations of these 28,695 cows. Final editing

was done by excluding daughters of sires with less than three recordings on CM and less than five recordings on SCC. This reduced the dataset to 94,781 lactations from 25,416 cows of different parities. Out of these 94,781 lactations, 41,828 lactations had recordings on both pathogen-specific CM and SCC, and 52,953 lactations had recordings on SCC only.

Because of the fixed sampling period of health data, variable lengths of DIM, number of days at risk during the study (days on trial; DOT), and the number of days in milk at start of the study (days at start; DAS) were present per lactation. Two variables were constructed to be able to adjust for DOT and DAS (De Haas et al., 2002b).

A pedigree file was constructed based on sires and maternal grandsires of cows in the data. The file contained 3,285 AI bulls with 2,073 sires plus 1,934 maternal grandsires (of which 1,068 were sires as well), and 346 fathers of the sires or maternal grandsires. The identification of the bull's mother was only included when she had two or more sons in the pedigree file, otherwise she was included as a base parent.

Definition of SCC patterns. Patterns of peaks in SCC distinguish between lactations with short or longer periods of increased SCC, and also between lactations with and without recovery within three or five test-day records. Upper and lower thresholds for SCC were set based on literature. Healthy and recovered cows were assumed to have less than 200,000 somatic cells/ml (Dohoo and Leslie, 1991). Infected cows were assumed to produce more than 500,000 cells/ml (Lam et al., 1997). Four patterns of peaks in SCC are described by De Haas et al. (2003). The first SCC pattern is referred to as a "quick recovery pattern" (P1), and describes a quick rise in SCC followed by an immediate decrease in SCC; i.e. consecutive test-day recordings of SCC had to be low-high-low. The second pattern is referred to as a "slow recovery pattern" (P2) and described a slower increase and decrease in SCC, but still with recovery; i.e. test-day recordings of SCC had to be low, higher, high, lower, and low again. The third pattern (P3) had no restrictions on SCC on the second and fourth test-day record, but the first one had to be low, the third one had to be high, and the fifth one had to be low again. The fourth pattern is referred to as the "no recovery pattern" (P4) and captured a longer increased SCC; i.e. one test-day with a low SCC recorded followed by four test-days with high SCC, so no recovery took place within four test-day recordings. Patterns of peaks in SCC could appear at each test-day up to 450 DIM. Each pattern of peaks in SCC was scored individually as a categorical trait, so when a SCC pattern was discovered in three or five consecutive test-day records, it was registered as 1, otherwise it was scored as 0.

In the current study, a fifth trait (P5) was defined which indicated whether any of the patterns of peaks in SCC was shown in the lactation or not, without specifying the pattern.

Since more than one pattern of peak in SCC could be present in a lactation, the sum of lactations with any of the SCC patterns present is less than the sum of lactations with presence of the individual SCC patterns.

The patterns were compared with the traditionally used LACSCS, based on the first 150 or 305 DIM. An average until 150 DIM was calculated if a cow had three or more recordings of SCC, otherwise a missing value was assigned. Similarly, missing values were replaced with averages until 305 DIM when SCC was measured at least six times. Both lactational averages were log transformed to somatic cell score ($SCS = \log_2(SCC/100{,}000)+3$), from now on referred to as SCS150 and SCS305.

Statistical analyses. AS-REML (Gilmour et al., 2002) was used to estimate variance components. Heritabilities were estimated in univariate analyses, using a linear model (Y) for SCS150 and SCS305, and using a logistic model (Logit(Y)) for P1, P2, P3, P4, P5 and pathogen-specific CM. Cows with missing values for SCS150, SCS305, SCC patterns or pathogen-specific CM were still included in the analyses. The model included random effects for sire and maternal grandsire (MGS) and an effect for animal, to account for the permanent animal effects across repeated lactations. The model used was:

$$Y \text{ or } Logit(Y) = \mu + \text{fixed effects} + S_{sire} + \tfrac{1}{2} S_{mgs} + PERM_{animal} + e$$

The random sire effect was identified by the subscripts for sire and MGS; S_{sire} and S_{mgs}, respectively. The sire effects were linked using the relationship matrix, and were assumed to be normally distributed with $var(S_{sire\ or\ mgs}) = \sigma^2_s$. Permanent animal effects contain environmental effects common to different lactations and genetic effects not covered by sire and MGS, like a dam-component, dominance, and Mendelian sampling terms. This was assumed to be normally distributed as well, with $var(PERM_{animal}) = \sigma^2_{Ea}$. For the logistic model, the residual variance (σ^2_ε) was fixed on 3.29; N(0, 3.29) (Gilmour et al., 2002). Residual covariances can only be estimated for those lactations with information on both pathogen-specific CM and SCC traits (i.e. P1, P2, P3, P4, P5, SCS150 or SCS305).

Fixed effects included were herd (with 274 levels), an interaction between year and season of calving (YS, with 43 classes), parity (with 4 classes, where the last class contains all parities ≥4), and the fraction of Holstein-Friesian genes (with 9 classes, for 0, 1/8, …, 8/8). For CM, polynomials of order 1, 2 and 4 were included for age at calving, DAS and DOT, respectively. For SCS150, SCS305, and SCC patterns, a polynomial of order 4 for age at calving was included. Order of the polynomials was established by stepwise inclusion of higher order regression coefficients (forward and backward elimination) till the estimated regression coefficient did not differ significantly from zero anymore.

Bivariate analyses were carried out to estimate correlations between SCC patterns and pathogen-specific CM, using a linear model for all traits. Combined linear and logistic models were also used, in which either the pathogens or the SCC patterns were treated as binary trait, and the other trait was assumed to be normal distributed. The estimated genetic correlations were similar to those originating from the complete linear model, and therefore only the estimated parameters from the complete linear model are shown. Fixed effects were the same as mentioned for the univariate analyses.

Calculation of genetic parameters. Genetic parameters were calculated from the estimated variance components. Additive genetic variance (σ^2_a) was calculated by multiplying sire variance by four. The phenotypic variance was the sum of (1) sire variance multiplied by 1.25, where 1.25 was included because MGS was fitted in the model separately, (2) permanent animal variance, and (3) residual variance. Division of additive genetic variance by phenotypic variance resulted in the heritability. Genetic and phenotypic correlations were estimated using the corresponding variances and covariances. Standard errors are provided by AS-REML, and give an indication of the accuracy of the estimates. Tests to assess statistical significance are not straightforward, since the distribution of the sampling variation is not normal.

Results

Heritabilities for patterns of peaks in SCC ranged from 0.01 to 0.06 (Table 5.1). Heritabilities were 0.07 (±0.01) for SCS150 and 0.08 (±0.01) for SCS305. Phenotypic correlations between two individual SCC patterns ranged from -0.03 to 0.50 (Table 5.2). Genetic correlations were high (0.74 to 0.99) between SCS150 or SCS305 and P2, P3, or P4 (Table 5.2), but low between P1 and SCS150 or SCS305 (i.e. 0.31 and 0.02, respectively). Genetic correlations were high between P1-P2, P1-P3 and P3-P4 (0.99, 0.97 and 0.88, respectively), whereas genetic correlations between P1-P4 and P2-P4 were moderate (0.31 and 0.62, respectively) (Table 5.2).

Phenotypic correlations between overall CM and LACSCS were positive; i.e. 0.26 for both SCS150 and SCS305, and ranged from 0.04 to 0.12 between overall CM and patterns of peaks in SCC (Table 5.3). Positive phenotypic correlations between pathogen-specific CM and SCS150 or SCS305 ranged from 0.06 to 0.20, indicating that LACSCS was higher in lactations with CM than in lactations without CM. Between pathogen-specific CM and SCC patterns the phenotypic correlations ranged from 0.01 to 0.10. These positive correlations indicated that on average the proportion of lactations with presence of patterns of peaks in SCC was higher

when considering all lactations with occurrence of pathogen-specific CM (n = 5,324) than when considering all lactations without occurrence of pathogen-specific CM (n = 36,504) (18 *vs.* 8%, 3 *vs.* 1%, 17 *vs.* 6%, and 10 *vs.* 3% for P1, P2, P3 and P4, respectively).

Table 5.1 *Number of lactations with presence of patterns of peaks in somatic cell count (SCC)*[1] *and with occurrence of pathogen-specific clinical mastitis, standard deviations for additive genetic (σ_a) and permanent animal (σ_{Ea}) effects, and heritability (h^2), all from univariate analyses, with standard errors in subscripts*

	No.	σ_a	σ_{Ea}	h^2
Pattern 1	7,540	0.17	0.32	$0.01_{0.01}$
Pattern 2	1,080	0.36	0.63	$0.03_{0.02}$
Pattern 3	5,951	0.16	0.34	$0.01_{0.01}$
Pattern 4	3,441	0.50	0.87	$0.06_{0.02}$
Any pattern	12,535	0.14	0.45	$0.02_{0.01}$
Clinical mastitis	5,324	0.39	0.51	$0.04_{0.01}$
Staphylococcus aureus	1,419	0.42	0.54	$0.05_{0.02}$
Coagulase-negative staphylococci	453	0.59	0.37	$0.10_{0.06}$
Escherichia coli	1,335	0.44	0.50	$0.05_{0.02}$
Streptococcus dysgalactiae	844	0.45	0.43	$0.06_{0.03}$
Streptococcus uberis	464	0.41	0.55	$0.05_{0.04}$
Other streptococci	637	0.25	0.47	$0.02_{0.03}$
Culture-negative samples	1,026	0.43	0.40	$0.05_{0.03}$
Other pathogens	675	0.44	0.19	$0.06_{0.04}$

[1] Pattern 1: quick recovery pattern (low-high-low SCC); pattern 2: slow recovery pattern (low-higher-high-lower-low SCC); pattern 3: low-no restrictions-high-no restrictions-low SCC; pattern 4: no recovery pattern (low-high-high-high-high SCC)); any pattern: presence of any of the earlier described patterns. Since more than one pattern of peak in SCC could be present in a lactation, the sum of lactations with any of the SCC patterns present is less than the sum of lactations with presence of the individual SCC patterns.

Genetic correlations between overall CM and patterns of peaks in SCC were stronger than the genetic correlations between CM and SCS150 or SCS305 (Table 5.4). In general, this holds for pathogen-specific CM as well, especially for cases of CM associated with *Staph. aureus*, CNS, *E. coli* and *Strep. dysgalactiae*. *Streptococcus uberis* and culture-negative CM showed similar genetic correlations with both patterns of peaks in SCC and SCS150 or SCS305. Genetic correlations of pathogen-specific CM with SCS150 were always stronger than correlations with SCS305 (Table 5.4).

Table 5.2 *Estimated genetic correlations below diagonal, and phenotypic correlations above diagonal from bivariate analyses between somatic cell count (SCC) patterns*[1] *and somatic cell score of lactational average cell counts up to 150 or 305 days in milk (SCS150 and SCS305, respectively), with their respective standard errors in subscripts*

	P1	P2	P3	P4	P5	SCS150	SCS305
Pattern 1		0.01 $_{0.003}$	0.50 $_{0.003}$	-0.03 $_{0.003}$	0.75 $_{0.003}$	0.21 $_{0.003}$	0.21 $_{0.003}$
Pattern 2	0.99 *		0.48 $_{0.003}$	0.24 $_{0.003}$	0.27 $_{0.003}$	0.10 $_{0.003}$	0.10 $_{0.003}$
Pattern 3	0.97 $_{0.122}$	0.99 *		0.09 $_{0.003}$	0.66 $_{0.003}$	0.23 $_{0.003}$	0.22 $_{0.003}$
Pattern 4	0.31 $_{0.239}$	0.62 $_{0.243}$	0.88 $_{0.174}$		0.48 $_{0.003}$	0.31 $_{0.003}$	0.35 $_{0.003}$
Any pattern	0.83 $_{0.130}$	0.99 *	0.99 *	0.97 $_{0.042}$		0.39 $_{0.003}$	0.41 $_{0.003}$
SCS150	0.31 $_{0.231}$	0.99 *	0.89 $_{0.126}$	0.84 $_{0.062}$	0.76 $_{0.081}$		0.88 $_{0.003}$
SCS305	0.02 $_{0.236}$	0.98 $_{0.146}$	0.74 $_{0.199}$	0.84 $_{0.061}$	0.67 $_{0.104}$	0.97 $_{0.012}$	

[1] Pattern 1 (P1): quick recovery pattern (low-high-low SCC); pattern 2 (P2): slow recovery pattern (low-higher-high-lower-low SCC); pattern 3 (P3): low-no restrictions-high-no restrictions-low SCC; pattern 4 (P4): no recovery pattern (low-high-high-high-high SCC); any pattern (P5): presence of any of the earlier described patterns.

* These genetic correlations were fixed at boundary.

Discussion

The objective of the study was to estimate genetic parameters for pathogen-specific CM, LACSCS, and patterns of peaks in SCC. This objective can be split up in three questions; i.e. (1) do these SCC patterns differ genetically from LACSCS, (2) is genetic selection on SCC patterns more effective to decrease the incidence of overall CM, than selection on LACSCS, (3) do these SCC patterns provide additional information for selection that aims to decrease susceptibility of pathogen-specific CM, in comparison with the information provided by LACSCS alone?

Table 5.3 *Estimated phenotypic correlations from bivariate analyses between pathogen-specific clinical mastitis, recorded in the first 450 days in lactation, lactation-average somatic cell scores, averaged over test-day records up to 150 and 305 days in milk (SCS150 and SCS305, respectively) and somatic cell count (SCC) patterns[1], with their respective standard errors in subscripts*

	P1	P2	P3	P4	P5	SCS150	SCS305
Clinical mastitis	$0.11_{0.01}$	$0.04_{0.01}$	$0.12_{0.01}$	$0.11_{0.01}$	$0.18_{0.01}$	$0.26_{0.01}$	$0.26_{0.01}$
Staphylococcus aureus	$0.03_{0.01}$	$0.03_{0.01}$	$0.05_{0.01}$	$0.10_{0.01}$	$0.10_{0.01}$	$0.18_{0.01}$	$0.20_{0.01}$
Coagulase neg. staphylococci	$0.03_{0.01}$	$0.02_{0.01}$	$0.02_{0.01}$	$0.05_{0.01}$	$0.05_{0.01}$	$0.09_{0.01}$	$0.08_{0.01}$
Escherichia coli	$0.08_{0.01}$	$0.02_{0.01}$	$0.07_{0.01}$	$0.02_{0.01}$	$0.10_{0.01}$	$0.10_{0.01}$	$0.09_{0.01}$
Streptococcus dysgalactiae	$0.03_{0.01}$	$0.02_{0.01}$	$0.05_{0.01}$	$0.05_{0.01}$	$0.07_{0.01}$	$0.12_{0.01}$	$0.11_{0.01}$
Streptococcus uberis	$0.04_{0.01}$	$0.01_{0.01}$	$0.04_{0.01}$	$0.05_{0.01}$	$0.07_{0.01}$	$0.08_{0.01}$	$0.09_{0.01}$
Culture-negative samples	$0.04_{0.01}$	$0.01_{0.01}$	$0.04_{0.01}$	$0.02_{0.01}$	$0.05_{0.01}$	$0.07_{0.01}$	$0.06_{0.01}$
Other pathogens	$0.03_{0.01}$	$0.01_{0.01}$	$0.04_{0.01}$	$0.03_{0.01}$	$0.06_{0.01}$	$0.08_{0.01}$	$0.08_{0.01}$

[1] P1: quick recovery pattern (low-high-low SCC); P2: slow recovery pattern (low-higher-high-lower-low SCC); P3: low-no restrictions-high-no restrictions-low SCC; P4: no recovery pattern (low-high-high-high-high SCC); P5: presence of any of the earlier described patterns.

SCC patterns *vs.* Lactational average SCS. Robertson and Lerner (1949) showed that parameter estimates from categorical traits using a linear model are frequency dependent, and should be transformed from the observable to the underlying scale for comparison purposes. Therefore, in this study a generalised linear model has been applied to the underlying liability scale, to estimate the (co)variance matrices for the categorically scored SCC patterns. Heritabilities for SCC patterns seem to be only slightly lower than those estimated for normally distributed LACSCS. However, if the estimates of heritabilities for the patterns of peaks in SCC had originated from analyses with a linear model, they would have been much lower than the estimates for LACSCS. Estimated heritabilities for SCS150 and SCS305 are low at approximately 0.08, but consistent with literature estimates. In a review of literature, Mrode and Swanson (1996) found a weighted h^2 estimate of 0.11 (±0.04).

Phenotypic correlations between two patterns of peaks in SCC were low. The strongest phenotypic correlations were estimated between P1 and P3 and between P2 and P3. The overlap between the definitions of P1 and P3 and of P2 and P3 was the main explanation for this higher phenotypic correlation. No restrictions were put on the second and fourth consecutive test-day for P3. When low SCC were recorded on these test-days, this overlapped with the definition for P1, when SCC between the lower and upper thresholds were recorded, this overlapped with the definition of P2. It is unlikely that both P1 and P2, or P1 and P4, or P3 and P4 are present in the same lactation, because it requires many test-day records to have both patterns present one after another. Phenotypic correlations between these SCC patterns were therefore low. The "slow recovery pattern" is less phenotypically correlated with both SCS150 and SCS305 than the "quick recovery pattern". This was not expected based on comparison of the calculated mean SCS150 and SCS305 in lactations with presence of P1 (3.98 and 4.26, respectively), and in lactations with presence of P2 (4.53 and 4.69, respectively). A possible explanation for the low phenotypic correlation might be the low incidence of P2, since in most lactations P2 = 0, and in only a few P2 = 1. Therefore, relatively, only a few values of SCS150 or SCS305 are associated with P2 = 1, and many values are associated with P2 = 0.

Genetic correlations between P1, P2 and P3 are high, implying these traits were similar to each other, which might be due to the existing overlap between the definitions of these patterns of peaks in SCC. The low genetic correlations between the "quick recovery pattern" and SCS150 or SCS305 imply that these are different traits. This can phenotypically be explained by a possible smaller effect of one single increase in SCC, compared to the effect of longer periods of increased SCC. As a result of this, selection for lower LACSCS will probably not accomplish the same effect as selection on less "quick recovery patterns" in a lactation. Ideally, a cow should recover quickly from an infection once she gets infected. It can be hypothesised that the "quick recovery pattern" belongs to cattle that recover quickly from an infection. Therefore, genetic selection for a lower presence of "quick recovery patterns" might improve the cow's abilities for a quick clearance of the infection.

Table 5.4 *Estimated genetic correlations from bivariate analyses between pathogen-specific clinical mastitis, recorded in the first 450 days in lactation, lactation-average somatic cell scores, averaged over test-day records up to 150 and 305 days in milk (SCS150 and SCS305, respectively) and somatic cell count (SCC) patterns[1], with their respective standard errors in subscripts*

	P1	P2	P3	P4	P5	SCS150	SCS305
Clinical mastitis	0.85 $_{0.18}$	0.99 *	0.99 *	0.76 $_{0.13}$	0.90 $_{0.08}$	0.74 $_{0.09}$	0.50 $_{0.13}$
Staphylococcus aureus	0.66 $_{0.27}$	0.98 $_{0.27}$	0.93 $_{0.19}$	0.50 $_{0.22}$	0.62 $_{0.20}$	0.53 $_{0.18}$	0.26 $_{0.19}$
Coagulase neg. staphylococci	0.99 *	0.99 *	0.97 $_{0.18}$	0.44 $_{0.30}$	0.89 $_{0.20}$	0.99 *	0.26 $_{0.25}$
Escherichia coli	0.92 $_{0.25}$	0.99 $_{0.29}$	0.85 $_{0.22}$	0.86 $_{0.14}$	0.90 $_{0.13}$	0.68 $_{0.17}$	0.54 $_{0.20}$
Streptococcus dysgalactiae	0.27 $_{0.38}$	0.65 $_{0.37}$	0.70 $_{0.31}$	0.40 $_{0.27}$	0.50 $_{0.25}$	0.28 $_{0.22}$	0.18 $_{0.23}$
Streptococcus uberis	0.49 $_{0.58}$	0.82 $_{0.60}$	0.99 *	0.68 $_{0.41}$	0.72 $_{0.42}$	0.73 $_{0.37}$	0.59 $_{0.40}$
Culture-negative samples	0.50 $_{0.31}$	0.59 $_{0.42}$	0.56 $_{0.34}$	-0.05 $_{0.25}$	0.31 $_{0.26}$	0.38 $_{0.20}$	0.29 $_{0.21}$
Other pathogens	0.55 $_{0.35}$	0.47 $_{0.38}$	0.55 $_{0.37}$	0.44 $_{0.24}$	0.48 $_{0.25}$	0.51 $_{0.19}$	0.45 $_{0.20}$

[1] P1: quick recovery pattern (low-high-low SCC); P2: slow recovery pattern (low-higher-high-lower-low SCC); P3: low-no restrictions-high-no restrictions-low SCC; P4: no recovery pattern (low-high-high-high-high SCC); P5: presence of any of the earlier described patterns.

* These genetic correlations were fixed at boundary.

Use of SCC patterns to decrease incidence of CM. The estimated genetic correlations fitted very well in the range of estimated genetic correlations between CM and SCC in other studies (0.3 to 0.9), which is reviewed by Mrode and Swanson (1996) and Emanuelson (1997). Correlations of 0.74 and 0.50 were estimated between CM and SCS in the first 150 and 305 DIM, respectively. This suggests that selection for lower SCS, especially during early lactation, also decreases the incidence of CM. Similar trends were reported by Emanuelson et al. (1988). Incidence of CM is higher in early lactation, as we have shown for this data (De Haas et al., 2002a), which might explain the higher correlation between CM and SCS150, compared with the correlation between CM and SCS305. In comparison with SCS150, the presence of any pattern of peaks in SCC is more strongly correlated with occurrence of CM. It seems that selection against any kind of deviation from the typical lactation curve for SCC would therefore to be more effective in decreasing the incidence of CM, than selection for lower LACSCS.

Use of SCC patterns to decrease incidence of pathogen-specific CM. Genetic correlations between pathogen-specific CM and patterns of peaks in SCC differed among pathogens, but were not as clear-cut as the phenotypic associations that were reported in an earlier study (De Haas et al., 2003). Genetic correlations between pathogen-specific CM and patterns of peaks in SCC were generally stronger than the correlations with SCS150 or SCS305, as was also observed for overall CM. This suggests that genetic selection purely on diminishing presence of peaks in SCC would decrease the incidence of cases of CM caused by all pathogens more effectively than selecting purely on lower LACSCS, but standard errors are large, so caution should be taken here.

The definitions of the currently analysed patterns of peaks in SCC were based on biological understanding of pathogens and the immune system of the cow. Apparently, these peaks do not distinguish clearly between resistance to certain pathogens on a genetic level. For example, from a biological point of view intramammary infections (IMI) with *Staph. aureus* can be characterised by a long duration and high SCC (Sears et al., 1990; Daley et al., 1991), which we confirmed at phenotypic level in a previous study (De Haas et al., 2003). In general, the estimated phenotypic correlations in this study were not high, but the highest for *Staph. aureus* was found with the "no recovery pattern". Unfortunately this trend was not confirmed by the genetic correlations. Instead, *Staph. aureus* CM shows the weakest genetic correlation with the "no recovery pattern" (P4), and not the strongest. On the other hand, *E. coli* CM are typically acute cases (Vaarst and Enevoldsen, 1997), which was also confirmed in the phenotypic study (De Haas et al., 2003). On the genetic level a strong correlation between *E. coli* CM and the "quick recovery pattern" (P1) was estimated. The higher correlation between P1 and *E. coli* CM is likely to come from a general stronger association between *E. coli* CM and all SCC patterns, rather than specific properties of P1.

So, maybe newly defined traits for SCC to be used as indirect traits in genetic selection programs should not only be based on biological backgrounds. Suggestions for other traits have been given in other studies (Heuven, 1987; Detilleux et al., 1997; Schepers et al., 1997).

Detilleux et al. (1997) concluded that analyses of SCC as candidate for selection against mastitis resistance could be improved by choosing better measures of SCC. These measures should contain all non-genetic factors that cause variation in SCC and methods of genetic epidemiology could be used as well. Depending upon the goal of the study, various ways of using SCC may be proposed for udder health surveillance. Examples they proposed were (1) proportion of test-day SCC above or below a certain limit, (2) direction and rate of change in test-day SCC, (3) time until SCC reach a given limit, (4) difference between observed SCC and SCC expected under healthy conditions, (5) area under (parts of) the lactation curve of SCC, (6) rolling averages, and (7) DIM that the increase in SCC happens. In relation to this, information on DIM of occurrence of CM could be taken into account as well, since recording increased SCC on test-days depend on (a) the day of occurrence of CM in relation to test-day recordings and (b) the duration of increased SCC as a result of pathogen-specific CM. The 'given limit' in the third suggestion of Detilleux et al. (1997) might, for instance, be the maximal recorded SCC during a lactation, which might be informative as to the mastitis-causing pathogen. On the one hand it was hypothesised that the higher peaks were associated with clinical *E. coli* mastitis, as these cases are known to be acute. However, in our study on the effects of pathogen-specific CM on the lactation curve for SCC we found that clinical *E. coli* mastitis did not have the strongest effect on SCC (De Haas et al., 2002a). Instead, cases of CM associated with either *Strep. dysgalactiae* or *Strep. uberis* resulted in the highest peaks in SCC. The method we have presented in the current study is a combination of the second and fourth suggestion of Detilleux et al. (1997). Including information on DIM at increase in SCC and DIM at occurrence of CM might improve the results.

Schepers et al. (1997) also provided alternative measures of SCC, based on the evaluation of the thresholds for IMI based on SCC. Twelve alternative SCC test statistics were calculated, divided in three groups: (1) three thresholds, for which identification of IMI was based on different fixed SCC values, (2) five thresholds, that were specific to parity, for which identification of IMI was based on the lactation curve of SCC, (3) four thresholds, for which identification of new IMI was based on deviation between current and previous samples in the same lactation. The use of SCC thresholds for specific parities and stages of lactation to detect IMI improved the quality of parameters only slightly over a fixed threshold of 200,000 cells/ml (Dohoo and Leslie, 1991). The third option of Schepers et al. (1997) is similar to the method used in the current study.

Finally, Heuven (1987) analysed test-day records of SCC to predict the presence of pathogens, and developed a method to identify abnormal observations of SCC, in order to exclude them from the dataset. An observation was considered to be abnormal on the basis of its deviation from the typical lactation curve. While using this exclusion method, he concluded that cows with either a high average SCC or a test-day with a high deviation from the typical lactation curve for SCC were more likely to be treated for CM. A single test-day with a high SCC recorded may not affect the lactation-average SCC much, whereas longer increased SCC will affect the lactation-average SCC eventually. Therefore, by selecting for lower (arithmetic) mean SCC during lactation the group of cows with a single high deviation from the typical lactation curve for SCC might be missed. However, these cows might still be more genetically susceptible to CM, as they might become infected more often, making it more likely that they would have elevated SCC on a test-day. In the current study the deviation from the typical lactation curve for SCC is taken into account, and therefore, the group of cows with one single high SCC recorded on a test-day can be identified.

In summary, selection for lower SCS, especially during early lactation, will decrease the incidence of CM, but in comparison with SCS150, the presence of any of the patterns of peaks in SCC is more strongly correlated with occurrence of CM. Genetic correlations between pathogen-specific CM and patterns of peaks in SCC should be interpreted with caution, because of high standard errors. However, the current results indicate a stronger genetic correlation between overall CM and presence of any pattern of peaks in SCC, and therefore encourage further research in patterns of peaks in SCC for genetic selection or mastitis control programs. Further optimisation includes increasing the accuracy of the estimated (co)variance matrices by enlarging the dataset for SCC. Other definitions of new traits for SCC (i.e. not based on biological backgrounds) as indirect traits in genetic selection programs should be considered as well.

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Chapter 6

Use of information about presence of somatic cell count patterns in herds to decrease incidence of clinical mastitis

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Abstract

Associations between clinical mastitis (CM) and presence of patterns of peak in somatic cell count (SCC) on herd level were determined in this study. Data on CM and SCC over a 12-month period were used from 274 Dutch herds. The dataset contained 29,719 (parts of) lactations from 22,955 cows of different parities. In total, 207,079 SCC test-days were recorded and 5,719 cases of CM; 1,561 cases were associated with environmental pathogens (ENV_CM), and 2,681 with contagious pathogens (CONT_CM). Definitions of patterns of peaks were based on three or five consecutive test-day recordings of SCC. They differentiated between short or longer periods of increased SCC, and also between lactations with and without recovery. Incidences of patterns of peaks in SCC varied among herds. Presence of SCC patterns were correlated with the incidence rate of CM. Herds with frequent presence of the quick recovery pattern had 2.5 times more chance to be classified as one of the 25% herds with highest incidence rates for CM. To be classified as one of the 25% herds with highest incidence rates for ENV_CM and CONT_CM they had 2.1 and 1.2 times more chance, respectively. Herds with frequent presence of the no recovery pattern had 1.1, 0.9 and 1.8 times more chance to be classified as a herd with high incidence rates of CM, ENV_CM and CONT_CM, respectively. As the incidences of SCC patterns were indicative for overall, environmental and contagious CM, the necessity to introduce pathogen-specific mastitis control programs in a herd could be determined based on the mean incidences of SCC patterns in that herd.

Keywords Somatic cell count, Patterns, Mastitis, Pathogens, Udder health management

Introduction

Mastitis can be caused by a large variety of pathogens. Mastitis-causing pathogens can be categorised depending on their aetiology into environmental and contagious pathogens (Fox and Gay, 1993; Smith and Hogan, 1993). *Escherichia coli* is the most important environmental pathogen (Smith and Hogan, 1993). The primary reservoir of environmental pathogens is the environment of the dairy cow. Exposure of udder quarters to the pathogen may occur at any time during lifetime, independent of the presence of infections in herd mates (Zadoks et al., 2001). Incidence rate of clinical mastitis (CM) associated with environmental pathogens is mostly related to housing conditions, hygiene and milking machine (Bartlett et al., 1992; Barkema et al., 1999). *Staphylococcus aureus* is the most important contagious pathogen (Fox and Gay, 1993), and its primary reservoir is the infected animal or udder quarter. Transmission is largely limited to the milking procedure and also to the milking machine (Schukken et al., 1991; Barkema et al., 1999). *Streptococcus dysgalactiae* is usually referred to as contagious (Hillerton et al., 1995), but most other streptococci are considered to be environmental (Smith et al., 1985; Pankey et al., 1987). However, contagious transmission of *Streptococcus uberis* has been reported (Zadoks et al., 2001), as well as persistent *E. coli* mastitis with recurrent cases (Döpfer et al., 1999; Bradley and Green, 2001). Zadoks (2002) discussed the option to represent the epidemiology of mastitis-causing pathogens as a sliding scale, where the balance of contagious and environmental transmission shifts gradually, rather than by a species-based dichotomy.

Understanding the epidemiology of a disease, including disease distribution and transmission, is important for the development of prevention and control programs. Procedures that may be very successful in control or eradication of contagious mastitis, may not be effective in the control of environmental mastitis, and vice versa (Neave et al., 1969; Fox and Gay, 1993; Smith and Hogan, 1993). A large number of control measures has been developed, and are combined in mastitis control programs (Park and Morgan, 1981; Oliver and Mitchell, 1984). The standard mastitis control program (Neave et al., 1969) decreases the prevalence of intramammary infections (IMI) with contagious pathogens (Neave et al., 1969; Hillerton et al., 1995), but it is less successful in preventing new cases of CM with environmental pathogens (Schukken et al., 1990; Lam et al., 1997; Barkema et al., 1999). Recommendations to control both contagious and environmental pathogens have been combined in a new ten-point mastitis control program, issued by the National Mastitis Council (2001).

So far, lactation-average somatic cell count (SCC) is generally used in mastitis control programs, but individual test-day recordings of SCC can be used instead. An effective use of SCC test-day records may be achieved by defining patterns of peaks in SCC during the lactation (De Haas et al., 2003). When these SCC patterns provide information on (1) the

pathogen-distribution on a farm, and (2) the success of therapy, these patterns may be better tools to use in mastitis control programs than lactation-average SCC. Health management advices can then be directed specifically on lowering the incidence rate of pathogen-specific CM, or shortening the duration of infection. Therefore, the aim of this study was to determine associations between incidence rates of pathogen-specific CM and of patterns of peaks in SCC on herd level, in order to investigate possibilities for giving health management advices based on the situation in the past year in a herd.

Material and Methods

Herds. Records on CM were available from a longitudinal prospective cohort study from December 1992 until June 1994 on 274 Dutch farms (Barkema et al., 1998). Lactating cows were housed in free-stall barns and milking parlours were double herringbone or two-sided open tandem. Herds participated in a milk recording system, and annual milk production quotas were between 300,000 and 900,000 kg. The national milk recording system (NRS, Arnhem, The Netherlands) provided information from the three- or four-weekly milk recordings. A record included national cow identification, breed, date of milk recording, date of calving, date of drying off, test-day milk yields and SCC (cells per ml). The main breeds were Holstein-Friesian, Dutch-Friesian and Meuse-Rhine-Yssel.

Sampling. Selection of herds and aseptic sampling procedures were described previously (Barkema et al., 1998). Data collection of milk samples of quarters with CM depended heavily on the willingness of farmers. Therefore, farmers were continuously encouraged, as described by Barkema et al. (1998). During the study period, farmers took milk samples from all quarters that, in their opinion, had clinical signs of mastitis. Examples of these clinical signs are abnormal texture and discoloration of the milk, swelling and discoloration of the udder, increased temperature or pain of the quarter. Quarter samples were stored in a freezer at the farm (at approximately -20°C) and were collected for bacteriological examination at intervals of six to eight weeks. Bacteriological culturing of milk samples was performed according to the standards of the National Mastitis Council (Harmon et al., 1990). Briefly, 0.01 ml was cultured, and for each culture, the number of colony-forming units of each of the bacterial species was counted. Collected data contained information on national cow identification number, date of mastitis occurrence, infected quarter, and result of bacteriological culturing of milk sample. Two groups of pathogens were defined: (1) contagious pathogens (CONT_CM) (Fox and Gay, 1993), consisting of *Staph. aureus*, coagulase-negative staphylococci and *Strep. dysgalactiae*, and (2) environmental pathogens (ENV_CM) (Smith and Hogan, 1993), consisting of *E. coli* and *Strep. uberis*. Intervals

between cases of CM in the same quarter had to be ≥14 days for a case to be included as a new case (Barkema et al., 1998).

Data selection. Originally, valid phenotypic records on CM and bacteriological characterisation were available on 47,563 lactations (De Haas et al., 2002b). For the present study, only the first 365 days of the study were subtracted from the dataset, because the aim of the study was to investigate the possibilities to give health management advices based on information from the past year in a herd. Therefore, the final dataset consisted of 29,719 (parts of) lactations from 22,955 cows of different parities. In total, 207,079 SCC test-days were recorded. All cases of CM in the year of observation were scored (n = 5,719), of which 1,561 were associated with environmental pathogens, and 2,681 with contagious pathogens. However, not every case of CM was covered by these two groups, since some bacteriological examinations were culture-negative (14.8% of all bacteriological examinations), or because the case of CM was associated with other pathogens, like *Corynebacterium bovis* or *Klebsiella* spp, or because the samples were contaminated (11.3% of all collected samples). In total, 15% of all cases of CM were associated with mixed cultures, and these cases were scored for both pathogens.

Trait definitions. Three groups of traits were defined; i.e. mastitis traits, newly defined traits for SCC and currently used traits for SCC. Traits were determined for individual cows, and summed per herd (n = 274).

Mastitis traits. Traits were determined for individual cows, and summed per herd (n = 274). Defined mastitis traits were total number of cases of CM, CONT_CM and ENV_CM. Incidence rates for CM, CONT_CM and ENV_CM were expressed per cow-day at risk. Cow-days at risk were calculated as the total number of days during the year of observation that a cow was in milk. The incidence rates were calculated per herd by dividing the sum of all cases of CM in a herd by the sum of all cow-days at risk in the herd (i.e. the number of days that a case of CM could have been observed).

Newly defined traits for SCC. Patterns of peaks in SCC distinguish lactations with short or longer periods of increased SCC, and also lactations with and without recovery from the increase in SCC within three or five test-day records. Upper and lower thresholds for SCC were set based on findings in literature. Healthy and recovered cows were assumed to have less than 200,000 somatic cells/ml (Dohoo and Leslie, 1991), and infected cows were assumed to produce more than 500,000 cells/ml (Lam et al., 1997). Therefore, test-day recordings of SCC were assumed to be low when the uncorrected SCC measured was

<200,000 cells/ml. When the uncorrected SCC was >500,000 cells/ml, the test-day recording of SCC was categorised as high. Intermediate SCC was defined as ≥200,000 and ≤500,000 cells/ml. It is expected that the risk of excluding early lactation data is minimal by setting these thresholds, because within a week after calving a cow is expected to have <200,000 cells/ml, and is it not very common that cows participate in the test-day recording shortly after calving (De Haas et al., 2002a).

The first SCC pattern is referred to as a "quick recovery pattern", and described a quick rise in SCC followed by an immediate decrease in SCC; i.e. consecutive test-day recordings of SCC had to be low-high-low. The second pattern is referred to as a "slow recovery pattern" and described a slower increase and decrease in SCC, but still with recovery; i.e. test-day recordings of SCC had to be low-intermediate-high-intermediate-low, respectively. The third pattern is referred to as a "no recovery pattern" and captured a longer increased SCC; i.e. one test-day with a low SCC recorded followed by four test-days with high SCC, so no recovery took place within four test-day recordings. The fourth pattern is referred to as a "shorter no recovery pattern" and captured an increased SCC for at least three consecutive test-days; i.e. four test-day recordings of SCC had to be low, high, high, and high. The fifth pattern is referred to as the "shortest no recovery pattern" and captured an increased SCC for at least two test-days; i.e. three test-day recordings of SCC had to be low, high, and high, consecutively.

Patterns were determined per cow, based on individual test-day records for SCC during the year of observation (De Haas et al., 2003). Only fully completed patterns were considered, and more than one pattern could have been determined per cow. Numbers of SCC patterns were 2,662, 361, 860, 1,082 and 2,353 for the pattern 1 – 5, respectively.

Incidence rate of SCC patterns refer to the ratio between the number of SCC patterns during the year of observation and the maximum feasible SCC patterns in the population at risk. The incidence rates were calculated per herd by dividing the sum of all observed SCC patterns of individual cows by the sum of the maximum feasible number of SCC patterns that could have been determined per cow. The maximum feasible number of SCC patterns depends on the number of consecutive test-day records of individual cows and on the length of the patterns. Firstly, the number of consecutive test-day records per cow was determined in the year of observation. Secondly, based on the length of the SCC patterns, it was calculated how many SCC patterns could have been observed per cow. For example, when a cow has 10 consecutive test-day records, and pattern 1 requires three test-day recordings, maximal 8 quick recovery patterns could have been observed. Lastly, all these maximum feasible number of SCC patterns per cow were summed up per herd.

Currently used traits for SCC. The patterns of peaks in SCC were compared with the currently used SCC traits for management in The Netherlands; i.e. number of new infections and the total number of test-days with SCC recorded above 250,000 cells/ml. A new infection was defined by consecutively a low (≤250,000 cells/ml) and high (>250,000 cells/ml) test-day recording of SCC. The incidence rate of new infections was calculated per herd by dividing the total number of new infections by the maximum feasible number of new infections that could have been determined in that herd. The proportion of high SCC was calculated by dividing the number of test-days with >250,000 somatic cells/ml by the total number of SCC test-day recordings in a herd.

Statistical analyses. Variation among herds was evaluated with histograms representing the number of herds in each class of incidence rates of pattern 1, 2 and 3. All herds contributed to the distribution of all three patterns of peaks in SCC. Besides, boundaries of incidence rates of all mastitis and SCC traits of the 10%, 25%, 50%, 75% and 90% best herds were determined by sorting the data for each trait separately.

Rank-order correlations. Spearman rank correlations between mastitis and SCC traits were estimated using PROC CORR in SAS (SAS/STAT®, 2001). The Spearman's rank-order correlation is a non-parametric measure that is calculated as the correlation of the ranks of the data. The purpose of this test was to compare herd rankings for all mastitis and SCC traits; i.e. whether or not herds ranked in the top of one of the SCC traits, were also ranked in the top of one of the mastitis traits. Mastitis traits were the incidence rates of CM, CONT_CM, and ENV_CM. The SCC traits were the incidence rates of patterns of peaks in SCC, and of new infections and the proportion of test-days with high SCC.

Effects of SCC traits on mastitis traits. In subsequent analyses, the effect of incidence rates of SCC traits on the incidence rates of mastitis traits was evaluated. Initially, incidence rates of the mastitis traits were described by fitting a smoothing spline function for different incidence rates of SCC traits in AS-REML (Gilmour et al., 2002). Usually, a spline function is used for smoothing data points and the function allows maximum flexibility and assumes no prescribed curvature. A cubic spline is a piecewise cubic function that is constrained so that the function and its first two derivatives are continuous at the breakpoints (knots) between one cubic segment and the next. The 'predict' statement in AS-REML was used to predict the incidence rates of the mastitis traits at different values of the incidence rates of the SCC traits. In order to vary the incidence rates of the mastitis traits over two patterns of peaks in SCC, an interaction between a spline describing incidence rate of CM as a function of pattern 1, and a spline describing incidence rate of CM as a function of the incidence rate of pattern 5 was modelled. The complete model estimated a function that described the incidence rate of CM at different incidence rates of both the quick recovery pattern and the

shortest no recovery pattern. It was decided to analyse the effect of these two SCC patterns because they were equally long and distinguished between a quick and no recovery from the infection.

Odds ratios. Final analyses were carried out by calculating odds ratios that describe associations between presence of (1) SCC patterns or (2) currently used SCC traits and the occurrence of CM, ENV_CM and CONT_CM in herds. Odds ratios are measures of associations, and indicate the change in risk. Statistical analyses were carried out to test if odds ratios differed from 1, using logistic regression in SAS (PROC LOGISTIC; SAS/STAT® (2001)).

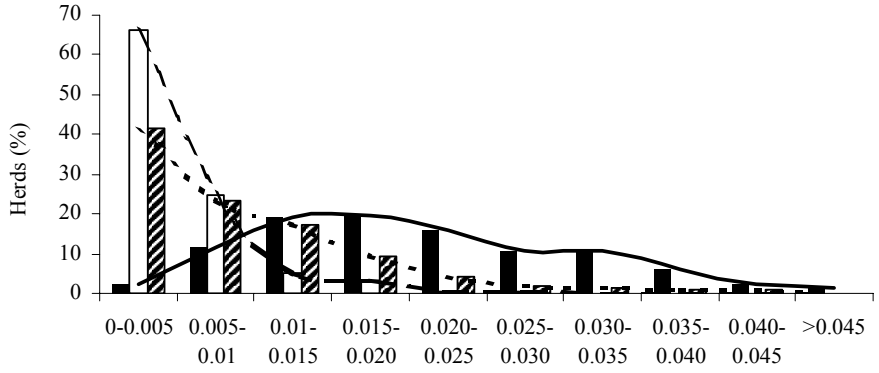

Figure 6.1 *Distribution of incidence rates of three SCC patterns of 274 herds stratified per pattern of peaks in SCC. Legend: quick recovery pattern (black bars), slow recovery pattern (white bars) and pattern with no recovery for at least four consecutive test-days (striped bars). The lines go over the tops of the bars, to show more visually how the data is distributed; the solid line goes over the black bars, the broken line goes over the white bars and the dotted line goes over the striped bars.*

Results

Distribution of the data. Incidence rates of patterns of peaks in SCC varied among herds, as the percentage of the 274 herds present in each class of incidence rate of the pattern of peaks differed per SCC pattern (Figure 6.1). The distribution of the incidence rate of the

slow and no recovery patterns seemed to be negative binomially distributed, and the incidence rate of the quick recovery pattern seemed to be normally distributed. The incidence rates of the quick recovery patterns ranged from zero to 0.045 (Table 6.1). As a result, maximal 59 quick recovery patterns will be present in a typical herd with 100 cows with each 15 test-day recordings in the year of observation (59 = 100 * (15-(3-1)) * 0.045). Differences in incidence rates of patterns of peaks in SCC existed between the 10%, 25%, and 50% best and worst herds (Table 6.1), which could be used as a basis for the classification of the herds.

Table 6.1 *Number of cases of clinical mastitis (CM) and of cases of CM associated with environmental (ENV_CM) or contagious (CONT_CM) pathogens, number of SCC traits[1], the mean incidence rates and the upper boundaries of the incidence rates of all mastitis[2] and SCC[3] traits in the 10%, 25%, 50%, 75%, and 90% best herds*

			Upper boundary of incidence rate				
	No.	Mean	10%	25%	50%	75%	90%
Mastitis traits							
CM	5,719	0.0006	0.0002	0.0003	0.0005	0.0008	0.0011
ENV_CM	1,561	0.0002	0.0000	0.0001	0.0002	0.0003	0.0005
CONT_CM	2,681	0.0003	0.0001	0.0002	0.0003	0.0006	0.0009
SCC traits							
Quick recovery	2,662	0.020	0.008	0.013	0.019	0.028	0.035
Slow recovery	361	0.004	0.000	0.000	0.003	0.006	0.009
No recovery	860	0.009	0.000	0.003	0.007	0.012	0.019
Shorter no recovery	1,082	0.011	0.000	0.004	0.009	0.015	0.022
Shortest no recovery	2,353	0.019	0.006	0.010	0.018	0.025	0.033
New infections	12,641	0.069	0.042	0.057	0.071	0.084	0.098
High SCC	41,253	0.195	0.089	0.135	0.193	0.251	0.298

[1] Quick recovery: low-high-low SCC; slow recovery: low-intermediate-high-intermediate-low SCC; no recovery: low-high-high-high-high SCC; shorter no recovery: low-high-high-high SCC; shortest no recovery: low-high-high SCC; new infection: low-high SCC.

[2] Number of cases of clinical mastitis per cow-day at risk.

[3] Number of SCC traits per maximum feasible number of SCC traits that could have been determined from the test-day recordings in a herd.

Associations among SCC traits. The estimated Spearman rank-order correlations among SCC traits indicated that herd rankings differed for the separate SCC traits (Table 6.2). Low correlations were estimated between the quick or slow recovery patterns and the other SCC traits, and moderate to high correlations were estimated between all three no recovery patterns and the other SCC traits. Even though the standard errors are large, this implies that herds that are ranked in the top for one of the no recovery patterns, are also ranked in the top for the other no recovery patterns, but not in the top for the quick or slow recovery pattern. Herds that are ranked high for the new infections are generally not ranked high for any of the other SCC traits.

Table 6.2 *Spearman rank-order correlations between SCC traits, with the standard errors in subscripts. The SCC traits are the incidence rates of the patterns of peaks in SCC[1], the incidence rate of new infections and the proportion of test-days with high SCC*

	P1	P2	P3	P4	P5	INF
Quick recovery						
Slow recovery	0.14 $_{0.06}$					
No recovery	-0.03 $_{0.06}$	0.15 $_{0.06}$				
Shorter no recovery	0.03 $_{0.06}$	0.17 $_{0.06}$	0.85 $_{0.02}$			
Shortest no recovery	0.31 $_{0.06}$	0.10 $_{0.06}$	0.65 $_{0.04}$	0.74 $_{0.04}$		
New infections	0.29 $_{0.06}$	0.24 $_{0.06}$	0.36 $_{0.06}$	0.44 $_{0.05}$	0.50 $_{0.05}$	
High SCC	0.07 $_{0.06}$	0.20 $_{0.06}$	0.63 $_{0.04}$	0.70 $_{0.03}$	0.71 $_{0.03}$	0.76 $_{0.03}$

[1] Quick recovery (P1): low-high-low SCC; slow recovery (P2): low-intermediate-high-intermediate-low SCC; no recovery (P3): low-high-high-high-high SCC; shorter no recovery (P4): low-high-high-high SCC; shortest no recovery (P5): low-high-high SCC; new infection (INF): low-high SCC.

Associations between mastitis and SCC traits. The estimated Spearman rank-order correlations between SCC patterns and mastitis traits in Table 6.3 indicated that the strongest association for the incidence rate of overall CM was found with the incidence rate of the quick recovery pattern (0.15), and this was mainly due to the strong association with ENV_CM (0.19). The incidence rates of all three no recovery patterns were positively associated with the incidence rate of CONT_CM. This implies that herds that are ranked to have a high incidence rate of the quick recovery pattern, are also ranked as herds with high occurrence of cases of overall and environmental CM. Herds that are ranked to have a high incidence rate of one of the no recovery patterns are ranked as herds with high occurrence of CONT_CM.

The estimated associations between the currently used SCC traits and the incidence rate of CONT_CM are reasonably high (Table 6.3). The association of the incidence rate of new infections with the incidence rate of ENV_CM was low, and the association with the proportion of test-day recordings >250,000 cells/ml was slightly negative (Table 6.3). This implies that ranking of herds based on the currently used traits for SCC is informative for ranking herds for CONT_CM, but not for ranking herds for ENV_CM.

Table 6.3 *Spearman rank-order correlations between mastitis and SCC traits, with standard errors in subscripts. Mastitis traits are the incidence rates of clinical mastitis (CM), CM associated with environmental pathogens (ENV_CM) and CM associated with contagious pathogens (CONT_CM). The SCC traits are the incidence rates of SCC patterns[1], the incidence rate of new infections and the proportion of test-days with high SCC*

	CM	ENV_CM	CONT_CM
Quick recovery	$0.15_{0.06}$	$0.19_{0.06}$	$0.02_{0.06}$
Slow recovery	$0.04_{0.06}$	$0.01_{0.06}$	$0.04_{0.06}$
No recovery	$0.08_{0.06}$	$-0.06_{0.06}$	$0.13_{0.06}$
Shorter no recovery	$0.04_{0.06}$	$-0.03_{0.06}$	$0.17_{0.06}$
Shortest no recovery	$-0.00_{0.06}$	$-0.02_{0.06}$	$0.19_{0.06}$
New infections	$0.09_{0.06}$	$0.01_{0.06}$	$0.20_{0.06}$
High SCC	$0.01_{0.06}$	$-0.06_{0.06}$	$0.19_{0.06}$

[1] Quick recovery pattern: low-high-low SCC; slow recovery pattern: low-intermediate-high-intermediate-low SCC; no recovery pattern: low-high-high-high-high SCC; shorter no recovery pattern: low-high-high-high SCC; shortest no recovery pattern: low-high-high SCC; new infection: low-high SCC.

Combined effect of two SCC patterns on incidence rate of mastitis. Combined information on the incidence rates of the quick and shortest no recovery pattern was informative for CM, ENV_CM and CONT_CM. The incidence rate of overall CM was increasing with increasing incidence rates of the quick recovery pattern, but not when the incidence rate of the shortest no recovery pattern was increasing (Figure 6.2a). Similar results were shown for ENV_CM; i.e. the highest incidence rate of ENV_CM was estimated when the incidence rate of the quick recovery pattern was high (Figure 6.2b). However, the incidence rate of CONT_CM was positively associated with the shortest no recovery pattern but not with the quick recovery pattern (Figure 6.2c). The highest incidence rate of CONT_CM was estimated when the incidence rate of the shortest no recovery pattern was high.

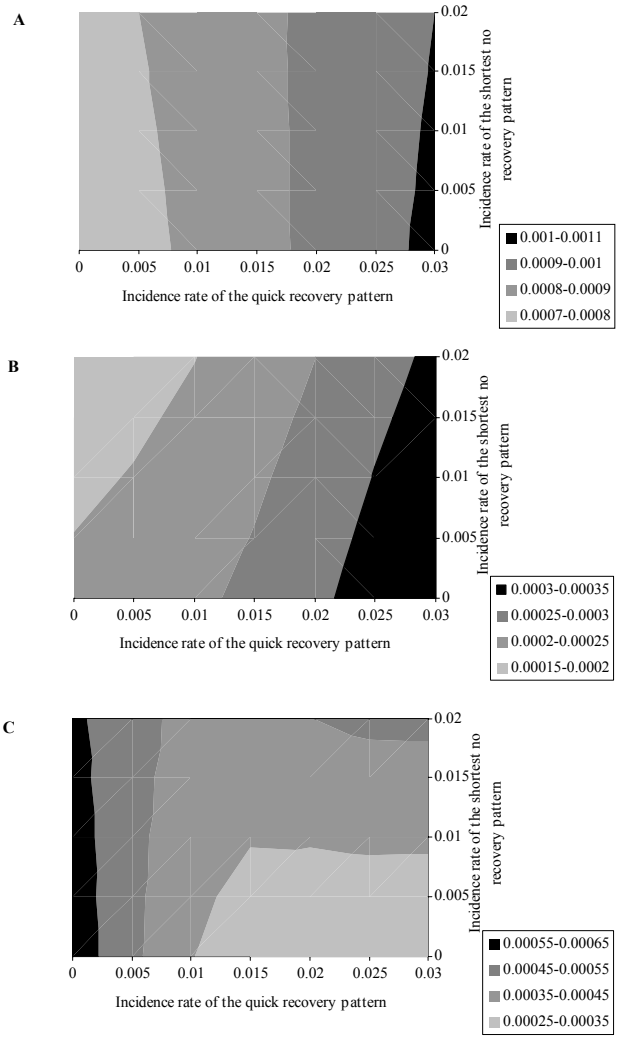

Figure 6.2a – 6.2c *Incidence rates of (a) overall clinical mastitis (CM), (b) CM associated with environmental pathogens, and (c) CM associated with contagious pathogens were modelled using two spline functions (and their interaction) for patterns of peaks in SCC, one function for the incidence rate of the quick recovery pattern and one for the incidence rate of the shortest no recovery pattern.*

Consequences of classification in 25% best or worst herds. Farmers are interested to know how the status of their herd relates to the status of other herds. Therefore, comparisons between the top and tail herds with respect to the incidence rates of SCC patterns were made, and linked to the risks for occurrence of CM, ENV_CM and CONT_CM. A herd classified in the top with respect to the lowest incidence rate of the quick recovery pattern had 1.7 times more chance to belong to the 25% herds with the lowest incidence rate of CM, compared to herds with higher incidence rates of the quick recovery pattern (Table 6.4). A comparable odds ratio (= 1.5) was estimated between the top 25% herds with respect to the incidence rate of the shortest no recovery pattern and the 25% herds with the lowest incidence rate of CM. On the other side, when a herd belonged to the worst herds with respect to the incidence rate for the quick recovery pattern, it had 2.1 times higher chances to be classified as a herd with high incidences rate of ENV_CM, compared to herds that were not classified as one of the worst herds for the quick recovery pattern (Table 6.4). A herd with high presence of the shortest no recovery pattern had high chances to be classified as a herd which belonged to the 25% herds with the highest incidence rate of CONT_CM (odds ratio = 1.8).

Table 6.4 *Calculated odds ratios indicating the chance of being classified as one of the best (b) or worst (w) 25% herds for occurrence of clinical mastitis (CM), CM associated with environmental pathogens (ENV_CM), and CM associated with contagious pathogens (CONT_CM) when classified in the best (b) or worst (w) 25% of the herds with respect to the mean incidence rate of two patterns of peaks in somatic cell count (SCC)[1] and of the currently used SCC traits[2]*

	P1 (b)	P1 (w)	P5 (b)	P5 (w)	INF (b)	INF (w)	HIGH (b)	HIGH (w)
CM (b)	1.7	0.4	1.5	0.5	2.5 *	0.8	1.6	1.1
CM (w)	0.8	2.5 *	0.9	1.1	0.6	1.1	0.7	0.7
ENV_CM (b)	1.8 *	0.6	0.8	1.0	1.3	0.9	1.0	1.1
ENV_CM (w)	0.6	2.1 *	1.4	0.9	1.1	0.8	1.2	0.4 *
CONT_CM (b)	1.5	0.4 *	1.7	0.5 *	2.5 *	0.6	1.8 *	0.7
CONT_CM (w)	1.4	1.2	0.6	1.8	0.7	1.4	0.4 *	1.4

[1] P1 (quick recovery pattern): low-high-low SCC; P5 (shortest no recovery pattern): low-high-high SCC.

[2] New infection (INF): low-high SCC, test-day recording with SCC >250,000 cells/ml (HIGH).

* Significantly different from 1, with $p < 0.05$.

The odds ratios for the currently used SCC traits indicate that they better distinguish between the 25% best and worst herds with respect to CONT_CM than with respect to ENV_CM. A herd with a low proportion of test-day recordings of SCC >250,000 cells/ml had also high chances to be classified as a herd which belonged to the 25% herds with the lowest incidence rate of CONT_CM (odds ratio = 1.8).

Discussion

Before starting a mastitis control program, it is important to analyse the herd situation. The currently used primary parameters are: (1) bulk milk somatic cell count, (2) percentage of cows with SCC >250,000 cells/ml per test-day, (3) percentage of cows with new infections, and (4) culling rate because of mastitis. Additionally, evaluation of the distribution of mastitis-causing pathogens can be informative. However, in 1997 only approximately 10% of the herds sampled cases of subclinical mastitis on a regular basis, and cases of CM were even less frequently sampled in The Netherlands (Sampimon, 1997). As a result, information of bacteriological sampling is unfortunately not often present. The results in the current study, however, indicate that presence of patterns of peaks in SCC provide information on the incidence of both CONT_CM and ENV_CM. The major advantage of using SCC patterns is that test-day recordings of SCC are freely available to all farmers that use SCC recording, suggesting that SCC patterns may facilitate the implementation of pathogen-specific mastitis control programs, and results are discussed against this background.

Comparison of SCC patterns with currently used traits. The estimated correlations in the current study show that incidence rates of the currently used SCC traits for management are mainly indicative for CONT_CM, but not so much for ENV_CM. This is in agreement with earlier statements that the standard mastitis control program is successful in decreasing the prevalence of IMI with contagious pathogens, but less successful in preventing new cases of CM with environmental pathogens (e.g. Hillerton et al. (1995)). However, patterns of peaks in SCC seem to provide information on both CONT_CM and ENV_CM. Namely, the incidence rate of the quick recovery pattern is stronger correlated to the incidence rate of ENV_CM than the currently used traits of SCC for management. The correlation between the incidence rate of the shortest no recovery pattern and the incidence rate of CONT_CM is similar to the ones between the currently used SCC traits for management and the incidence rate of CONT_CM. Therefore, information on presence and absence of a combination of patterns of peaks in SCC might be indicative for the occurrences of CONT_CM and ENV_CM.

In the current study, it is shown that occurrences of ENV_CM are mainly indicated by presence of quick recovery patterns, and to a lesser extent by absence of no recovery patterns. This was expected, since cases of ENV_CM are known to be typical acute cases (Vaarst and Enevoldsen, 1997). On the other hand, occurrences of CONT_CM seem to be mainly indicated by an absence of the quick recovery pattern, and to a lesser extent by the presence of the shortest no recovery pattern. One explanation could be that cases of CONT_CM are often characterised by a long duration and high SCC (Sears et al., 1990; Daley et al., 1991), and therefore, presence of quick recovery patterns is unlikely.

Application of SCC patterns. Based on the mean incidence rates of SCC traits in a herd, the necessity to introduce pathogen-specific mastitis control programs in a herd, consisting of adequate guidelines to control the predominant type of mastitis, can be determined. To be able to present adequate guidelines, it is necessary to have an overview of all test-day recordings of SCC in a herd to be able to calculate incidence rates. Besides, comparing herds, classifying them in the top or tail of the herds, might be informative. Most progress might, therefore, be obtained when the patterns of peaks in SCC are applied by national milk recording systems, because it is relatively easy for them to provide an overview on each test-day of the incidence rates of SCC patterns in a herd in the past year. Improvement of the possibilities for using the information for management advices, might be achieved by publishing the incidence rates of patterns of peaks in SCC for heifers and older cows separately. Differences in patterns of peaks in SCC around a case of pathogen-specific CM between heifers and older cows were namely shown in an earlier study (De Haas et al., 2002a). An even further optimisation could be achieved by publishing only the incidence rates of SCC patterns present in early lactation as in an earlier study we showed that 90% of most pathogen-specific cases of CM had occurred before 200 days in milk, using a similar dataset (De Haas et al., 2002a). Therefore, analysing only the first part of the lactation might increase the incidence rates of SCC patterns as patterns are expected to occur around the cases of CM. Furthermore, it might be worthwhile to look at other definitions of SCC patterns in which the patterns of peaks in SCC are, for example, analysed in a quantitative manner; i.e. analysing SCC and CM as two traits that are continuous over time and using a variance and covariance matrix to describe the association between variation in SCC and incidence of CM. However, to investigate this, an even more advanced technique (like a random regression model) has to be implemented. With random regression test-day models, deviations from the standard curve are analysed. These deviations from the standard curve can then be specified more precisely as either the rate of increase or decrease in SCC, or the slope of increase or decrease in SCC. These two parameters are derivations of either the first or second half of the SCC patterns, and therefore, the patterns investigated in the current study are a foretaste for quantitative parameters of SCC to be defined in further continuation studies.

When the incidence rates of SCC patterns of the past year are provided on each test-day, this results in a rolling average. Changes in the status of the udder health in a herd could then be easily detected, and management advices could be directed specifically on changing herd risk factors related to either CONT_CM or ENV_CM, depending on what the predominant type of mastitis in that herd is. Effects of changes in management on the status of the udder health in a herd might become visible as a change in the rolling average.

Conclusions

The major advantage of using patterns of peaks in test-day recordings of SCC, over bacteriological culture of cases of CM, is that they are freely available to all farmers that use SCC recording. Information on both the quick and shortest no recovery pattern could therefore easily be included in mastitis control programs that aim to capture the full scope of environmental and contagious mastitis-causing pathogens. However, it should be kept in mind that the division of pathogens into environmental and contagious is not absolute. Next to the ability to distinguish between environmental and contagious mastitis, the SCC patterns provide additional information on, for example, the incidence of (spontaneous or therapeutic) cure. This suggests that the quick and shortest no recovery pattern are useful as basic tools for health management advices. Based on the incidence rates of SCC traits in a herd, the necessity to introduce pathogen-specific mastitis control programs, consisting of adequate guidelines to control the predominant type of mastitis in that herd, can be determined. Effects of changes in the udder health management on the status of the udder health in a herd could be easily evaluated when the incidence rates of SCC traits are provided as a rolling average on each test-day by the national milk recording system.

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Chapter 7

General discussion

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The aims of this study were threefold. Firstly, to improve our understanding of the effect of genetic selection on lower lactation-average somatic cell count (SCC) on the occurrence of pathogen-specific clinical mastitis (CM) (Chapter 2). Secondly, to identify the phenotypic associations between the occurrence of pathogen-specific CM and the presence of patterns of peaks in SCC (Chapter 3 and 4). Thirdly, to investigate the possible use of SCC patterns for genetic selection and management while aiming to decrease the incidence of pathogen-specific CM (Chapter 5 and 6). In the current chapter, results from the preceding chapters are discussed with a focus on the practical implementations. Separate sections discuss the implementations for genetic selection strategies and pathogen-specific mastitis control programs. Furthermore, two additional topics will be discussed; i.e. are the results on CM also applicable for subclinical mastitis (SCM), and whether or not SCC can be too low?

Consequences of genetic selection

In the last decade, there is a growing concern in the western society about excessive use of antibiotics in food animals. Moreover, the costs of treatment and non-preventative veterinary care contribute to economic losses for the farmer. Therefore, breeding for genetic resistance to infectious diseases becomes more attractive (Knap and Bishop, 1999). Breeding for disease resistance is a challenging approach. The progress per generation might be small and costly, but it is heritable, and therefore cumulative over generations. Enhanced resistance to disease is stable under natural selection, and therefore artificial selection for disease resistance should be stable and sustainable (Stear et al., 2001). Moreover, enhanced disease resistance will result in reduced transmission of an infectious agent from one host to the entire herd, or from herd to herd (Bishop and Stear, 1997). Interaction among individuals in a population is an important factor for the transmission of the infectious agent (Koopman and Longini, 1994), and when infected animals are not able to infect other susceptible animals, because of the enhanced disease resistance, the infectious agent cannot persist in the population and will fade out (Diekmann et al., 1990).

Current selection strategy. Selection for improved udder health is of primary importance in dairy cattle populations, and it is now included in selection indices in most countries (VanRaden, 2002). The strongest emphasis to udder health is given in Scandinavian countries, with a relative weight of milk yield compared to mastitis of 1 in Norway, and of 0.42, 0.30 and 0.35 in Denmark, Finland and Sweden, respectively (Interbull, 1996). A joint use of a direct measure, such as CM incidence, and SCC as an indirect measure is used for the breeding value estimation in Denmark, Finland and Sweden (Heringstad et al., 2000; Pedersen et al., 2002), while Norway only uses information on CM (Heringstad et al., 2000;

Svendsen and Ranberg, 2000). Other countries in the world select for udder health using indirect measures, such as SCC, udder conformation and milking speed. The ways to analyse SCC differ largely between countries (Mark et al., 2002); i.e. lactation-average based *vs.* test-day repeatability *vs.* random regression test-day models. At the start of this project, lactation-average SCC was analysed for genetic evaluation in The Netherlands, but starting at May 2003 a random regression test-day model is applied.

Traditionally, genetic selection strategies aim to simultaneously increase milk yield and decrease lactation-average SCC. The assumption crucial to select for lower SCC is that cows with lower SCC are less likely to have an intramammary infection (IMI) and, therefore, are more resistant to IMI, and to CM and SCM as well. Selection for decreased SCC would then reduce susceptibility to (sub)clinical mastitis (Colleau and Le Bihan-Duval, 1995). One concern of using lactation-average SCC in genetic selection has been that it would not only select for a reduced susceptibility to CM, but also against the possibilities of a cow to respond to an IMI. Experimental challenge studies indicated that elevated SCC before infusion protects against infection by (other) mastitis-causing organisms (Schalm et al., 1964; Carroll et al., 1973; Kehrli and Shuster, 1994; Schukken et al., 1994). However, the estimated genetic relationships, assuming linearity between CM and SCC, suggest that SCC should be decreased to the lowest possible value, at least within the range covered by the population mean and the genetic variance in their population (Emanuelson et al., 1988; Philipsson et al., 1995; Mrode and Swanson, 1996; Rupp and Boichard, 2001; Chapter 2).

Table 7.1 *Results of bacteriology of clinical mastitis samples in the last 50 years*[1]

	1950	1970	1980	1990	2000
No. of submitted samples	1,221	634	1,726	4,962	5,777
% Culture-positive	56.6	51.4	58.4	51.0	70.5
% Culture-positive for:					
Staphylococcus aureus	14.7	47.2	21.0	30.4	22.7
Coagulase-negative staphylococci	-[2]	-[2]	-[2]	-[2]	6.8
Escherichia coli	2.6	0.6	25.1	31.6	26.1
Streptococcus agalactiae	44.5	23.0	5.2	2.9	0.4
Streptococcus dysgalactiae	4.1	3.1	18.8	13.2	8.1
Streptococcus uberis	7.2	14.1	8.2	10.7	17.1
Other streptococci	14.1	10.1	2.5	3.1	0.7
Other pathogens	12.8	1.9	19.2	8.1	18.1

[1] Source: Dutch Animal Health Service, Deventer, The Netherlands (Sol, 2002).
[2] not recorded.

Pathogen-specific selection index. Another concern of using lactation-average SCC in genetic selection might follow from the large variety of pathogens that can cause CM. At the beginning of this project, it was not very well known whether selection for lower SCC improves resistance to only some or to all pathogens, since the aetiology of each mastitis-causing pathogen is different. In Chapter 2, we reported low heritabilities for pathogen-specific CM, and a range of genetic correlations between pathogen-specific CM and lactation-average SCC was estimated. These results are similar to results of earlier studies by Rogers et al. (1995) and Nash et al. (2000). The estimated heritabilities and genetic correlations indicate that selection for lower lactation-average SCC might cause a shift in occurrence of mastitis-causing pathogens. The expected correlated responses showed that current selection indices (using milk yield and lactation-average SCC) will be effective in reducing the incidence of *E. coli* and *Strep. uberis*, but less effective in reducing the incidence of *Staph. aureus* and *Strep. dysgalactiae*, even with a large relative weight on lactation-average SCC in the selection index (Chapter 2). Therefore, a decreasing importance of *E. coli* and *Strep. uberis*, and an increasing importance of *Staph. aureus* and *Strep. dysgalactiae* could be expected, when observing the distribution of mastitis-causing pathogens in the past decades.

The Dutch Animal Health Service reported the distribution of mastitis-causing pathogens during the past 50 years (Table 7.1). *Streptococcus agalactiae* has nearly been eradicated, while the proportion of *Staph. aureus* and *E. coli* isolations has increased. Also *Strep. uberis* became more prevalent, and the proportion of *Strep. dysgalactiae* isolations increased until the 80's and decreased from then on. It is important to consider, however, that these phenotypic data from cases of CM reported by the Dutch Animal Health Service might be biased, and are not a random sample of the cases of CM in The Netherlands, as cases of CM are not frequently sampled (Sampimon, 1997). Also, it is important to consider that next to the change in genetic level, changes in environment and farm management play an important role in the change of the phenotypic recordings.

Opportunities to improve genetic strategies

Evidence from immunology and genetics has been reviewed, demonstrating that selective breeding of genetically based resistance is likely to modify the cow's susceptibility to pathogens (Detilleux, 2002). The challenge is to integrate effectively the information from both fields into existing breeding programs. This is in line with our hypothesis that traits for SCC should be based on biological understanding of pathogens and the immune system of the cow (Chapter 4). These traits can then be used as indirect traits in genetic selection programs that aim to decrease the genetic susceptibility for the full scope of mastitis-causing pathogens (Chapter 5).

Improving the mastitis index. An index method combines information into a single figure that gives an optimal selection criterion. Optimal is defined as 'most accurate' or 'giving the highest selection response when selecting for it'. To capture the full scope of mastitis-causing pathogens, an index which does not only include the lactation-average SCC, but a combination of SCC traits might be necessary. To analyse this, we have calculated accuracies of several selection indices, consisting of all possible combinations of SCC traits; i.e. patterns of peaks in SCC and lactation-average SCC. The patterns of peaks in SCC were the quick, slow and no recovery pattern (Chapter 4). Lactation-average SCC was based on the first 150 or 305 days in milk (DIM); i.e. SCS150 and SCS305, respectively, where SCS = $\log_2$(SCC/100,000)+3 (Chapter 5). Due to the large number of bivariate analyses carried out in Chapter 5, some of the eigenvalues of the correlation matrix were negative and therefore made positive. The correlation matrix was recalculated using the eigenfunctions (Hill and Thompson, 1978). In this new positive definite matrix, 61% of the correlations had changed by less than 0.10, and 90% by less than 0.20. Comparisons were made between sires with 10, 100, 1,000 or 10,000 daughters.

Table 7.2 *Accuracies of selection indices including one trait[1] aiming to decrease incidence rates of clinical mastitis (CM), and CM associated with either environmental (ENV_CM), or contagious pathogens (CONT_CM), based on sires with 100 or 10,000 daughters*

	100 daughters			10,000 daughters		
	CM	ENV_CM	CONT_CM	CM	ENV_CM	CONT_CM
Quick recovery pattern	0.363	0.332	0.329	0.794	0.727	0.722
Slow recovery pattern	0.564	0.607	0.563	0.854	0.919	0.852
No recovery pattern	0.552	0.629	0.403	0.708	0.807	0.517
Any pattern	0.515	0.559	0.440	0.881	0.957	0.754
SCS150	0.567	0.650	0.488	0.728	0.834	0.627
SCS305	0.409	0.563	0.277	0.499	0.686	0.337

[1] Quick recovery pattern: low-high-low SCC; slow recovery pattern: low-higher-high-lower-low SCC; no recovery pattern: low-high-high-high-high SCC; any pattern: presence of any of the earlier described patterns; SCS150 or SCS305: somatic cell score of test-day recordings up to 150 or 305 days in milk, respectively.

Overall mastitis. When the breeding goal is to decrease the incidence rate of overall CM, the mastitis indices are developed to optimally predict the cow's susceptibility to cases of CM. For first proven sires with 100 daughters, it was more effective to select for lower SCC in early lactation, than to select for lower lactation-average SCC based on 305 DIM, to decrease the incidence of overall CM (Table 7.2). However, similar progress was achieved by selecting on less presence of slow or no recovery patterns. When selecting well-proven sires, selection against any deviation from the typical lactation curve for SCC was more effective to decrease the incidence of CM, than selection for lower lactation-average SCC.

Table 7.3 *Accuracies of selection indices including a combination of SCC traits[1] aiming to decrease the incidence rate of overall clinical mastitis, based on sires with 10, 100, 1,000 or 10,000 daughters*

	10 daughters	100 daughters	1,000 daughters	10,000 daughters
P1 – P2	0.257	0.615	0.848	0.892
P1 – P3	0.289	0.636	0.871	0.937
P1 – SCS150	0.275	0.615	0.864	0.936
P1 – SCS305	0.224	0.511	0.799	0.896
P2 – P3	0.306	0.662	0.853	0.887
P2 – SCS150	0.325	0.659	0.819	0.855
P2 – SCS305	0.285	0.593	0.812	0.872
P3 – SCS150	0.317	0.626	0.744	0.759
P3 – SCS305	0.281	0.561	0.688	0.709
P1 – P2 – P3	0.329	0.706	0.905	0.947
P1 – P2 – SCS150	0.332	0.688	0.877	0.943
P1 – P2 – SCS305	0.298	0.637	0.849	0.897
P1 – P3 – SCS150	0.329	0.676	0.892	0.954
P1 – P3 – SCS305	0.301	0.639	0.872	0.939
P2 – P3 – SCS150	0.351	0.689	0.854	0.902
P2 – P3 – SCS305	0.322	0.662	0.895	0.958
P1 – P2 – P3 – SCS150	0.361	0.723	0.906	0.955
P1 – P2 – P3 – SCS305	0.339	0.706	0.916	0.958

[1] P1: quick recovery pattern (low-high-low SCC); P2: slow recovery pattern (low-intermediate-high-intermediate-low SCC); P3: no recovery pattern (low-high-high-high-high SCC); SCS150 or SCS305: somatic cell score of test-day recordings up to 150 or 305 days in milk, respectively.

When the index consisted of lactation-average SCC plus one SCC pattern, most progress in decreasing the incidence rate of overall CM was made with a downwards selection on the quick recovery pattern and SCS150 (Table 7.3). Comparable progress was achieved with a combination of the quick and no recovery pattern included in the index. When more SCC traits were included in the index, an index including the slow and no recovery pattern and SCS305 was most accurate.

Table 7.4 *Accuracies of selection indices including a combination of SCC traits[1] aiming to decrease the incidence rates of clinical mastitis associated with either environmental (ENV_CM), or contagious pathogens (CONT_CM), based on sires with 100 or 10,000 daughters*

	ENV_CM		CONT_CM	
	100 daugh.	10,000 daugh.	100 daugh.	10,000 daugh.
P1 – P2	0.639	0.924	0.602	0.869
P1 – P3	0.687	0.953	0.502	0.795
P1 – SCS150	0.676	0.963	0.535	0.827
P1 – SCS305	0.616	0.949	0.405	0.777
P2 – P3	0.733	0.971	0.593	0.852
P2 – SCS150	0.733	0.926	0.622	0.854
P2 – SCS305	0.691	0.919	0.565	0.905
P3 – SCS150	0.716	0.869	0.514	0.634
P3 – SCS305	0.671	0.818	0.406	0.521
P1 – P2 – P3	0.761	0.987	0.631	0.872
P1 – P2 – SCS150	0.747	0.964	0.645	0.869
P1 – P2 – SCS305	0.715	0.949	0.603	0.909
P1 – P3 – SCS150	0.745	0.985	0.563	0.830
P1 – P3 – SCS305	0.717	0.979	0.502	0.797
P2 – P3 – SCS150	0.771	0.979	0.626	0.857
P2 – P3 – SCS305	0.747	0.989	0.595	0.937
P1 – P2 – P3 – SCS150	0.788	0.988	0.652	0.872
P1 – P2 – P3 – SCS305	0.772	0.989	0.634	0.952

[1] P1: quick recovery pattern (low-high-low SCC); P2: slow recovery pattern (low-intermediate-high-intermediate-low SCC); P3: no recovery pattern (low-high-high-high-high SCC); SCS150 or SCS305: somatic cell score of test-day recordings up to 150 or 305 days in milk, respectively.

Pathogen-specific mastitis. When the breeding goal is to decrease the incidence rate of pathogen-specific CM, the mastitis indices are developed to optimally predict the cow's susceptibility to pathogen-specific cases of CM. Estimated correlations showed that it was hard to reduce the susceptibility to specific mastitis-causing pathogens (Chapter 5). We have tried to increase the accuracies of the estimated (co)variance matrices by enlarging the dataset of SCC. To achieve this, the NRS (Arnhem, The Netherlands) provided an additional dataset including test-day recordings of SCC at 1,000 herds. The patterns of peaks in SCC were determined based on the method described in Chapter 4. Data was edited by selecting only those cows, which were genetically related to the cows in the health dataset via the sire or maternal grandsire. Directions of the estimated genetic correlations between pathogen-specific CM and patterns of peaks in SCC and standard errors did not change much. Hence, although it confirmed our results in Chapter 5, it was surprising that the standard errors were still high with this large amount of data. This illustrates the problem to estimate genetic parameters with low incidence traits, like pathogen-specific CM.

To increase the incidence of pathogen-specific CM, we grouped certain pathogens together. Mastitis-causing pathogens can be categorised depending on their aetiology into environmental and contagious pathogens (Fox and Gay, 1993; Smith and Hogan, 1993). The primary reservoir of environmental pathogens is the environment of the dairy cow and *E. coli* is the most important environmental pathogen (Smith and Hogan, 1993). *Staphylococcus aureus* is the most important contagious pathogen (Fox and Gay, 1993), and its primary reservoir is the infected animal or udder quarter.

Two new breeding goals were defined that aimed to decrease the incidence rates of cases of CM associated with (1) environmental pathogens (ENV_CM), or (2) contagious pathogens (CONT_CM). The indices consisting of only one SCC trait showed that selection against any kind of deviation from the typical lactation curve for SCC was most effective to decrease the incidence rate of ENV_CM (Table 7.2). However, to decrease the incidence rate of CONT_CM, selection against presence of the slow recovery pattern was most effective. When one SCC pattern was included in the index, in addition to lactation-average SCC, most progress in decreasing the incidence rate of ENV_CM can be made by downwards selection on the quick recovery pattern and SCS150 (Table 7.4). For CONT_CM, an index consisting of the slow recovery pattern and SCS305 was most accurate. When two patterns of peaks are added in the index, a combination of the slow and no recovery pattern and SCS305 was most accurate for both ENV_CM and CONT_CM, which coincides with the index for overall CM. Introducing pathogen-specific mastitis indices can be helpful in addressing herd-specific problems, by increasing the genetic resistance of cows to pathogen-specific CM. Note, genetic selection is a slow process as progress per generation is small, but it results in a permanent change in the genetic composition of the dairy herd (Shook, 1989). Changing the environment of the cow, however, is easier to realise and effects are directly visible.

Further optimisation. Test-day models provide a better adjustment for systematic environmental effects (Reents et al., 1995). This seems especially important for SCC, because test-day recordings of SCC might have been affected by infection pressure on a certain date, a milking machine that was working poorly, or any other effect of the sample date. Test-day models offer the opportunity of using all available information simultaneously while accounting for environmental effects at their origin. Furthermore, non-linear mixed models could be used as well to analyse SCC (Rodriguez-Zas et al., 2000b). Between-cow variation of trajectories of the lactation curve of SCC suggests that lactation curve parameters could be modified by genetic selection in some advantageous manner (Rodriguez-Zas et al., 2000a).

At the start of this project, lactation-average SCC was analysed for genetic evaluation in The Netherlands, but starting at May 2003 a random regression test-day model is applied. By applying the random regression test-day model, deviations from a standard curve are analysed. Now the question arises how the patterns of peaks in SCC can be combined with the use of the random regression test-day model. One way to implement this, is to specify the deviations from a standard curve more precisely to either the rate of increase or decrease in SCC, or the slope of increase or decrease in SCC. These two parameters are derivations of either the first or second half of the SCC patterns. Therefore, the patterns investigated here are in line with the introduction of a random regression test-day model for SCC. In fact, the random regression test-day model might prove to be a good tool to estimate breeding values for patterns.

Pathogen-specific mastitis control programs

Before starting a mastitis control program, it is important to analyse the herd situation. The currently used primary parameters are: (1) bulk milk somatic cell count (BMSCC), (2) percentage of cows with SCC >250,000 cells/ml per test-day, (3) percentage of cows obtaining SCC >250,000 cells/ml, and (4) culling rate because of mastitis. Additionally, evaluation of the distribution of pathogens of both CM and SCM can be informative. However, in 1997 only approximately 10% of the herds sampled cases of SCM on a regular basis, and cases of CM were even less frequently sampled in The Netherlands (Sampimon, 1997). As a result, information of bacteriological sampling is unfortunately not often performed. Information on pathogen distribution could, however, be provided by using SCC test-day recordings for management (Chapter 4 and 6).

Improvement of current mastitis control program. In Chapter 4 and 6, we showed that a different use of SCC increases the predictive value of the bacteriological status of a cow. Patterns of peaks in SCC enable better distinction between ENV_CM and CONT_CM than the currently used primary parameters. The primary parameters were informative for CONT_CM though; i.e. herds with high proportions of test-day recordings of SCC >250,000 cells/ml had higher incidence rates for CONT_CM compared to herds with lower proportions. However, the primary parameters were not indicative for ENV_CM, but the presence of patterns of peaks in SCC was indicative for ENV_CM. Herds with frequent presence of the quick recovery pattern had higher incidence rates for ENV_CM compared to herds with less presence of this pattern of peaks in SCC. On the other hand, herds where the no recovery pattern was found frequently had higher incidence rates for CONT_CM compared to herds with lower incidences of this SCC pattern. Therefore, information on both the quick and no recovery pattern should be used to capture the full scope of environmental and contagious mastitis-causing pathogens. Next to the ability to distinguish between ENV_CM and CONT_CM, the SCC patterns provide additional information on, for example, the incidence of (spontaneous or therapeutic) cure. This suggests that the quick and no recovery pattern are useful as basic tools for health management advices.

A fictitious example of the current Dutch information sheet that is distributed after a test-day recording is shown in Appendix 7.1. Part A at the form contains information on SCC per group of cows (i.e. parity, lactation stage or production level) and per herd. For these traits, only the information from the current test-day is used. The provided information per group of cows is: (1) the number of cows in lactation, (2) the number of cows with SCC >250,000 cells/ml, and (3) the number of cows that obtain a SCC >250,000 cells/ml. For the traits in part B at the form, the averages from all test-days in the past year is shown. The provided information is the percentage of cows with SCC >250,000 cells/ml of all tested cows on a test-day, and BMSCC. In part C, individual cow-information on production traits, SCC and projected 305-day yields is provided. Information on the quick and no recovery pattern could be added in each part of the distributed form, and the advantages and disadvantages of two options are presented below.

The first option is to present the absolute number of quick or no recovery patterns that has been completed at the current test-day. Patterns of peaks have to be established per individual cow (presented at part C of the form), and then summed up for the cows grouped per lactation stage or parity to give an overview (presented at part A of the form). An illustration of how this could be presented on the information sheet that is distributed after a test-day recording, is shown in Appendix 7.1. Specifying the presence of SCC patterns per lactation stage or parity might have the advantage that it shows more precise which cows are most susceptible; i.e. at what lactation stage or in which age-class. This helps to put special attention on cows that are at risk. However, as shown in Chapter 6, the maximum number of patterns of peaks in SCC was 2,662 and this was a total based on 207,079 test-

day recordings of SCC on 274 farms. Therefore, in a particular herd, the number of cows that show one of the SCC patterns will be low, and thus not very informative.

The second option is to present the incidence rates of SCC pattern as calculated in Chapter 6 in part B of the form. Presenting the incidence rate of the quick and no recovery pattern in the previous 6 or 12 months will result in a rolling average, because the period refers to changes on each test-day. A period of 12 months is preferable, because all annual returning effects, like changes in housing, feeding and weather, will then be included in the period referred to with the rolling average. The incidence rates of the quick and no recovery pattern are indicative for the incidence rate of cases of CM associated with either environmental or contagious pathogens, respectively (Chapter 6). Introduction of pathogen-specific mastitis control programs in a herd, consisting of adequate guidelines to control the predominant type of mastitis are necessary when the incidence rates of the patterns of peaks in SCC are larger than 0.01 (i.e. the median calculated in Chapter 6). Pathogen-specific mastitis control programs aim to reduce the number of new IMIs, and to limit the duration of the existing infections.

Pathogen-specific herd risk factors. By providing a rolling average of the quick and no recovery pattern on each test-day, changes in the status of the udder health in a herd can be detected. Therefore, management advices can be directed specifically on changing herd risk factors related to either CONT_CM or ENV_CM. Furthermore, effects of these changes on the status of the udder health in a herd can easily be evaluated based on the provided incidences of the SCC patterns on each test-day. Several herd risk factors can be specifically linked with cases of ENV_CM, and others are mainly associated with cases of CONT_CM. Housing, nutrition and machine milking are important factors influencing the incidence of ENV_CM (Bartlett et al., 1992; Barkema et al., 1999). Poor sanitation, use of tie stalls, and no use of individual cloths are associated with increased incidence rates of ENV_CM. Herd characteristics that are associated with higher incidence rates of CONT_CM are mainly related to the milking procedure and to the milking machine as well (Schukken et al., 1991; Barkema et al., 1999). Frequent cleaning of the milking system regulator, low-line milking systems and applying disinfectant solution to the teat after milking ('teat dipping') are effective in decreasing the number of cases of CONT_CM (Hutton et al., 1991; Lam et al., 1997; Middleton et al., 2001).

Cure of infection. The interpretation of the SCC patterns can also be linked to the success of the treatment of CM. Cure of IMI, that has resulted in (sub)clinical mastitis, can occur spontaneous or therapeutic. Whether or not spontaneous cure occurs, is partly a pathogen-specific effect, next to the effect of the immune system of the cow. From some pathogens it is known that spontaneous cure hardly ever occurs; e.g. *Staph. aureus*. *Staphylococcus aureus* invades into mammary epithelial (Dego et al., 2002), and IMIs with *Staph. aureus* are therefore often characterised by a long duration and high SCC (Sears et al., 1990; Daley

et al., 1991; Chapter 3). Cure from other pathogens, like *E. coli*, can occur spontaneous. Clinical *E. coli* mastitis is mostly of short duration (Vaarst and Enevoldsen, 1997; Chapter 3), and bacteria are readily eliminated from the mammary gland. However, in addition to acute *E. coli* mastitis, persistent *E. coli* mastitis with recurrent cases does occur as well (Döpfer et al., 1999), but as mentioned in Chapter 1, Zadoks (2002) stated that classifying mastitis-causing pathogens as purely environmental or contagious is often an oversimplification. Epidemiology of mastitis-causing pathogens is better represented by a sliding scale, where the balance of contagious and environmental transmission shifts gradually, than by a species-based dichotomy.

Whether or not the therapeutic cure is successful depends on the treatment strategy applied in a herd. Extended antibiotic therapy does improve the bacteriological cure rate of clinical *Staph. aureus* mastitis (Sol et al., 2000; Gillespie et al., 2002), and a beneficial effect of simultaneous application of intramammary and intramuscular treatment was found as well (Owens and Nickerson, 1990). Therefore, management decisions concerning the chosen approach of therapy influence the success of the antibiotic therapy. These management decisions can be supported by knowledge of the incidence rates of the quick and no recovery patterns. A high incidence rate of quick recovery patterns might imply a high rate of cures of infection; either spontaneous or therapeutic. Whereas, a high incidence rate of the no recovery pattern might indicate that the treatment strategy in a herd is not successful. The pattern after the rise in SCC is affected by the type of therapy. In our study we analysed cows with CM which were treated with antibiotics. Therefore, it was expected that SCC generally would decrease sharply after the rise, resulting in many quick recovery patterns, and high associations between all mastitis-causing pathogens and the quick recovery pattern. However, other SCC patterns than the quick recovery pattern were also found, and associations between pathogen-specific cases of CM and specific patterns of peaks in SCC were found as well (Chapter 4 and 6). Probably even stronger associations would have been found between mastitis-causing pathogens and SCC patterns when the pattern after the rise in SCC would not have been affected by the type of therapy of the cow. The fact that associations between mastitis-causing pathogens and SCC patterns were still found even though the cows were treated with antibiotics, encourage the use of patterns of peaks for management purposes.

Subclinical mastitis

For most farms, SCM is economically the most important form of mastitis because of long-term reductions in milk yield (El Bayomi and Mahmoud, 1987; Barillet et al., 2001). Subclinical mastitis is characterised by apparently normal milk, with an increase in SCC due to the influx of leukocytes (Sears and McCarthy, 2003), and therefore, SCC provides a good

quantitative estimate of the degree of infection. Subclinical mastitis can also be characterised by the results of bacteriological culturing of milk samples, but unfortunately, information of bacteriological sampling is not often present (Sampimon, 1997). As a result, SCC is the most important source of information to determine the prevalence of SCM. In our studies, we analysed associations between cases of CM and several SCC traits, but the question rose whether these SCC traits are informative for cases of SCM as well.

Genetics. The genetic correlation between SCM and lactation-average SCC is 0.50 (El Bayomi and Mahmoud, 1987), which is comparable with the ones estimated between CM and lactation-average SCC (Mrode and Swanson, 1996; Emanuelson, 1997; Chapter 2). The moderately high genetic correlation between SCM and SCC indicates that selecting for lower SCC would increase the genetic resistance to overall SCM (Rupp and Boichard, 2001). Only recently, research is interested in estimating genetic parameters on pathogen-specific SCM (Van Dorp, 2003). Low heritabilities are estimated for SCM associated with either environmental or contagious pathogens; i.e. 0.018 and 0.005, respectively. These estimates are slightly lower than the estimates for pathogen-specific CM we presented in Chapter 2. Trends in genetic correlations between pathogen-specific SCM and milk yield or SCC are similar to what we have reported between pathogen-specific CM and milk yield or SCC (Chapter 2). Cases of SCM associated with environmental pathogens have a strong correlation with SCC and are even negatively correlated with milk yield, although not significantly different from zero (Van Dorp, 2003). Cases of SCM associated with contagious pathogens are strongly correlated with milk yield, and weakly with SCC (Van Dorp, 2003). Therefore, selection on lower lactation-average SCC will probably cause a shift in the main pathogens causing SCM, equally to the shift in pathogens causing CM (Chapter 2). As a result, contagious pathogens will become more prevalent as mastitis-causing pathogens, unless a different definition of SCC can provide information on SCM as well. Although we can only speculate on this, the mastitis index including the slow and no recovery pattern and SCS305 (Table 7.4) might also be effective to decrease the prevalence of both environmental and contagious SCM. Subclinical mastitis is namely known to cause long increased SCC, resulting in presence of no recovery patterns, and high SCS305.

Management. Bulk milk SCC is one of the currently used primary parameters for udder health management, as it is determined on a regular basis. Values of BMSCC are only indicative of SCM, and monthly test-day recordings of SCC, obtained from individual cows, are necessary to define the prevalence and incidence of SCM (Ruegg, 2003). The patterns of peaks in SCC defined in Chapter 4 are based on test-day recordings of SCC. These SCC patterns can identify cows with a no recovery pattern and this might be informative for the

prevalence of SCM, since cases of SCM are known to cause long increased SCC. However, other reasons for a long increased SCC (i.e. low rate of cure, or occurrence of pathogen-specific CM) have been discussed in previous paragraphs of this chapter as well. Therefore, only indications of the prevalence of SCM in a herd can be given by the presence of no recovery patterns, and additional information on bacteriological culturing is necessary to diagnose SCM with certainty.

Can somatic cell count be too low?

Somatic cells play an important role in combating udder infections. As mentioned briefly earlier in this chapter, there is a concern that a too low SCC could lead to a higher susceptibility to CM (Coffey et al., 1986). Experimental challenge studies indicated that a slightly elevated SCC before infusion protects against infection by (other) mastitis-causing pathogens (e.g. Kehrli and Shuster, 1994). The question "Is there a risk that too low SCC leads to more cases of pathogen-specific CM?" is therefore of great biological importance, and is re-iterated on many conferences and meetings. Results from our studies are discussed below against this background.

Making a comparison at herd level shows that the incidence of clinical *Staph. aureus* and *Strep. dysgalactiae* mastitis was higher in herds with high BMSCC than in herds with low BMSCC. On the other hand, herds with low BMSCC have high incidences of CM associated with environmental pathogens or with culture-negative samples (Eberhart et al., 1982; Erskine et al., 1988; Hogan et al., 1989; Sischo et al., 1993; Barkema et al., 1998). These results may indicate that pathogens are associated with either different baseline levels for SCC or different duration of cases of CM, as both of them may affect BMSCC. However, the level of BMSCC does not reflect the mean SCC of cows with a low SCC in that herd. Clearly, these studies do therefore not answer the question whether or not a low SCC at cow level implicates an increased risk of CM. To study this risk, we have evaluated SCC at cow level in Chapter 3, and the results in this study do not support that cows with low SCC are more susceptible for CM. For none of the pathogens was SCC before a case of CM below the level of lactations without both CM and SCM. For heifers, SCC was virtually the same before a case of clinical *E. coli* mastitis, and for multiparous cows slightly higher. This, together with the increased SCC both before and after a case of CM associated with all pathogens, suggests that avoiding high SCC is important to reduce CM.

One concern of using lactation-average SCC in genetic selection has been that it would not only select for a reduced susceptibility to IMI, but also against the possibilities for a cow to respond to an IMI. The estimated genetic relationships suggest that daughters of sires that transmit the lowest SCC had the lowest number of cases of overall CM (Philipsson et al.,

1995; Mrode and Swanson, 1996; Rupp and Boichard, 2001; Nash et al., 2000). Therefore, SCC should be decreased to the lowest possible value, at least within the range covered by the population mean and the genetic variance in their population, and the theory that selection for the lowest SCC will result in dairy cattle that are unable to respond to IMI is not supported, because if such were the case, the lowest SCC would be associated with a higher number of cases of CM. Hence, these studies do not answer the question if there is a higher risk for clinical *E. coli* mastitis when using bulls that transmit low SCC. Relationships among pathogen-specific CM and sire transmitting abilities for SCC have only recently been determined (Nash et al., 2000; Nash et al., 2002). These authors concluded that selection for lower SCC may also improve genetic resistance to pathogen-specific CM, as daughters of sires that transmit higher SCC showed higher incidence rates of all pathogen-specific CM (including *E. coli*). This is in line with the genetic correlations between SCC and pathogen-specific CM we have estimated in Chapter 2.

Environmental mastitis is generally of shorter duration than contagious mastitis (Fox and Gay, 1993; Smith and Hogan, 1993). Therefore, Shook (1993) hypothesised that selection for lower SCC may not improve resistance to ENV_CM, because with monthly test-day recordings the elevated SCC due to ENV_CM may not be detected. However, this seems contradictory to the fairly strong positive genetic correlation between SCC and ENV_CM we have estimated (Table 7.2). One explanation could be that exposure to environmental pathogens occurs daily, and the less resistant cows may become infected more often, or have a longer lasting increased SCC. As a result, the less resistant cow may be more likely to have elevated SCC on the test-day, which is also affirmed by the determined association between ENV_CM and the slow or no recovery pattern (Chapter 4 and 5). Another explanation might be that a case of clinical *E. coli* mastitis causes such a strong increase in SCC, that the predominant source of variation in SCC is due to cows having CM or not, rather than having a high or low SCC prior to infection. This suggests that genetic selection on diminishing presence of peaks in SCC would decrease the incidence of pathogen-specific CM without risking an increased susceptibility of the cows to IMI because of a too low SCC.

General conclusions

Selecting for lower lactation-average SCC causes a shift in the importance of the main mastitis-causing pathogens. Therefore, there is a growing importance for defining new traits for SCC, that are based on biological understanding of pathogens and of the immune system of the cow. These traits can be used for genetic selection and for mastitis control programs that aim to reduce the incidence of the full scope of mastitis-causing pathogens.

Genetic selection against any kind of deviation from the typical lactation curve for SCC was more effective to decrease the natural susceptibility to mastitis-causing pathogens, than selection for lower lactation-average SCC. An index including the slow and no recovery pattern, plus the lactation-average SCC (305d) was most accurate to decrease the incidence of overall, environmental and contagious CM, and most likely to decrease the prevalence of SCM as well.

The quick and no recovery pattern are useful as basic tools for health management advices. These patterns of peaks in SCC can namely distinguish between cases of CM associated with either environmental or contagious pathogens, whereas the currently used primary traits are indicative for contagious, but not for environmental mastitis. Presenting the incidence of both the quick and the no recovery pattern as a rolling average on the forms of the milk recording is of additional value. Management advices can then be directed specifically on changing herd risk factors related to either environmental or contagious mastitis, and effects of these changes on the status of the udder health in a herd can be easily evaluated.

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Appendix 7.1 *A fictitious example of the current Dutch information sheet that is distributed after a test-day recording*

C Mastitis
Cell Count Rd 2
1010 DR Cowburgh

Date test-day 03/07/03
Date laboratorium 05/07/03

A

Somatic cell count information

Group	number	% >250	n new		quick recovery	no recovery
< 60 dim	5	0	0		0	0
61 - 120 dim	9	1	0		1	0
121 - 180 dim	15	0	0		2	2
180 - 305 dim	34	5	1		1	0
> 305 dim	10	2	1		0	0
heifers	28	1	0		2	0
2nd parity	13	0	0		2	1
multiparous	32	7	2		0	1
herd	**73**	**8**	**2**		**4**	**2**

B

% cows with SCC >250,000 cells/ml

date	23/08	15/09	05/10	27/10	16/11	08/12	03/01	24/01	16/02	06/03	27/04	07/06	03/07
% >250	11	16	17	19	20	19	12	13	10	9	10	9	12

Bulk milk somatic cell count

date	11/07	10/08	14/09	03/10	07/11	07/12	02/01	08/02	13/03	03/04	01/05	05/06	10/07
BMSCC	104	180	260	275	277	161	162	126	117	89	159	127	126

Incidence rate of quick recovery pattern (*1,000)

date	23/08	15/09	05/10	27/10	16/11	08/12	03/01	24/01	16/02	06/03	27/04	07/06	03/07
inc. quick	0	1	1	2	5	8	12	10	7	6	8	10	8

Incidence rate of no recovery pattern (*1,000)

date	23/08	15/09	05/10	27/10	16/11	08/12	03/01	24/01	16/02	06/03	27/04	07/06	03/07
inc. no	0	1	1	3	4	5	6	8	9	8	10	9	7

C

Animal		Daily production				SCC	Projected 305d yields							
name		kg	%	%	kg	*1,000	calving date	DIM	kg	%	%	kg	kg
recording no.		milk	fat	prot	f+p		age lactnr		milk	fat	prot	fat	prot
Theodora		16	4.58	3.40	15	152	26/03/03	**99**	**9,701**	**4.58**	**3.40**	**444**	**329**
123456789							2.01 1		7,290	4.32	3.23	315	235
Maria		24	4.46	3.63	22	21	21/02/03	**132**	**5,136**	**4.72**	**3.63**	**242**	**186**
123456789							4.05 3		7,776	4.62	3.65	359	284
Roelina		20	4.89	3.76	18	128	14/06/02	**384**	**5,209**	**4.70**	**3.41**	**245**	**178**
123456789							2.03 1		6,375	4.72	3.48	301	222
Hermania		24	4.92	3.56	22	164	27/09/02	**279**	**5,242**	**4.56**	**3.41**	**239**	**179**
123456789							3.02 2		7,843	4.64	3.48	364	273
Ynta		34	3.42	2.84	17	74	23/10/02	**253**	**822**	**3.63**	**2.94**	**30**	**24**
123456789							4.05 3		6,348	3.63	3.16	230	201
Juda		17	4.81	3.96	16	123	12/05/03	**52**	**5,834**	**4.40**	**3.60**	**257**	**210**
123456789							3.08 2		6,790	4.44	3.65	301	248
Silvia		23	4.11	3.33	20	33	8/08/02	**329**	**1,449**	**4.43**	**3.35**	**64**	**48**
123456789							2.03 1		6,247	4.37	3.50	273	219
Froukje		27	5.56	3.05	29	297	18/02/03	**135**	**465**	**5.56**	**3.05**	**26**	**14**
123456789							5.10 4		6,905	5.54	3.39	383	234
Lutina		20	4.84	3.71	18	1008	8/02/03	**145**	**4,044**	**4.62**	**3.45**	**187**	**139**
123456789							2.05 1		6,255	4.66	3.54	292	221
Kaatje		30	4.37	3.29	22	13	20/03/03	**105**	**2,060**	**4.80**	**3.39**	**99**	**70**
123456789							4.03 3		7,167	4.61	3.45	330	247
Marcolina		25	3.76	3.49	19	21	7/11/02	**238**	**8,253**	**4.05**	**3.39**	**335**	**280**
123456789							2.05 1		8,149	4.06	3.39	331	276
Franske		17	4.36	4.12	14	360	15/05/03	**49**	**9,786**	**3.86**	**3.34**	**377**	**327**
123456789							2.01 1		7,450	3.75	3.21	279	239
Lisa		19	7.23	4.41	22	85	6/04/03	**88**	**4,124**	**6.59**	**4.23**	**272**	**174**
123456789							3.11 2		6,226	6.66	4.30	415	268
Bartha		21	4.21	3.50	16	54	3/10/02	**273**	**4,127**	**4.54**	**3.53**	**188**	**146**
123456789							3.05 2		6,388	4.42	3.54	283	226
Mariska		16	5.34	3.65	15	17	24/11/02	**221**	**2,758**	**5.03**	**3.43**	**139**	**95**
123456789							1.11 1		4,693	5.04	3.53	237	166
Karola		32	3.95	3.38	26	51	11/07/02	**375**	**1,471**	**4.09**	**3.32**	**60**	**49**
123456789							4.04 3		7,176	4.09	3.58	293	257
…													

Summary

Mastitis is an inflammation of one or more quarters of the udder of a cow, generally caused by bacteria, and is one of the major diseases in dairy herds. It induces economic costs, mainly consisting of discarded milk, increased health care costs and reduced milk quality. Mastitis also contributes to consumer concerns regarding animal welfare and regarding the impact of use of antibiotics in animals on efficacy of antibiotics for human health. Visible signs of an inflammatory response of the udder coincide with clinical mastitis (CM). Examples of these visible signs are abnormal texture and discoloration of the milk, and swelling and discoloration of the udder. Subclinical mastitis (SCM) is not clinically visible, and laboratory techniques such as bacteriological culture and measurement of somatic cell count (SCC) are needed to detect infection and inflammation. Over 100 different bacteria can cause mastitis, but most of the economic losses are associated with species of the coliform bacteria, the staphylococci and the streptococci (for more details, see **Chapter 1**).

Somatic cells are indicators of both resistance and susceptibility of cows to intramammary infections. Milk somatic cells consist of several cell types, including leukocytes, macrophages, lymphocytes and a smaller percentage of epithelial cells. In healthy lactating mammary glands, macrophages are the predominant cell type, whereas leukocytes are the major cell population during early inflammation. The leukocytes play a protective role against infectious diseases in the bovine mammary gland as they phagocyte and destroy infectious agents (for more details, see **Chapter 1**).

At the start of the project, little was known about the genetic variation of incidences of mastitis caused by different pathogens and whether selection for yields and SCC affects all pathogens equally or not. Therefore, the aim of the study presented in **Chapter 2** was to quantify genetic variation for overall and pathogen-specific CM was and to estimate genetic correlations with milk production and SCC. Records on CM were available from a study carried out from December 1992 till June 1994 on 274 Dutch farms (Barkema et al., 1998. J. Dairy Sci. 81: 411-419). The national milk recording system (NRS, Arnhem, The Netherlands) provided information from a three- or four-weekly milk recording system. During the study period, farmers took milk samples from all quarters that, in their opinion, had clinical signs of mastitis. Samples were stored in a freezer at the farm (at approximately -20°C) and were collected for bacteriological examination at intervals of six to eight weeks. Analysed pathogens were *Staphylococcus aureus*, coagulase-negative staphylococci (CNS), *Escherichia coli*, *Streptococcus dysgalactiae*, *Streptococcus uberis* and other streptococci. In total, 47,563 lactations of 28,695 cows of different parities were recorded for at least one day during the study. The estimated heritability for overall CM was 0.04, and similar heritabilities for the pathogen-specific CM were estimated. Genetic correlations with milk yield and SCC differed according to pathogen. The expected correlated responses showed that current selection practices (using milk yield and SCC) will be effective in reducing the incidence of *E. coli* and CNS but less effective in reducing the incidence of *Staph. aureus* and *Strep. dysgalactiae*, even with a large relative weight for SCC in the selection index.

Somatic cells are always present in the milk. A typical lactation curve for SCC starts off high shortly after parturition, decreases in the first 50 days in milk to a lowest point, and increases slowly from then on towards the end of the lactation. So far, lactation-average SCC is generally used in mastitis control programs and for genetic improvement of udder health. However, this lactation-average SCC ignores the deviations from the typical lactation curve that can be caused by CM. Using test-day recordings of SCC, instead of lactation-average SCC, increases the possibilities to identify variation among test-days as indicators of the incidence of mastitis-causing pathogens. Pathogen-specific effects on SCC during lactation were investigated in **Chapter 3**, by analysing the pattern of SCC before and after a case of pathogen-specific CM, relative to the lactation curve for lactations without both CM and SCM. The effect of CM on the lactation curve for SCC was large but differed per pathogen. Somatic cell count always remained elevated after the occurrence of pathogen-specific CM, although the effect was smaller when the interval between the occurrence of CM and the day of sampling was larger. Before a case of clinical *E. coli* mastitis occurred, SCC was close to the SCC of lactations without both CM and SCM, and after the case of CM had occurred, SCC returned rather quickly to a low level again. Similar curves were found for lactations with cases of CM associated with culture-negative samples. Before a case of clinical *Staph. aureus* mastitis occurred, SCC was already high, and it remained high after the occurrence. Effects of cases of CM associated with *Strep. dysgalactiae*, *Strep. uberis,* and other streptococci on the lactation curve for SCC were comparable. They showed a continuous increase in SCC until the case of pathogen-specific CM occurred, and afterwards SCC stayed on a higher level. From this, we concluded that deviations from the typical curve of SCC during lactation characterise mastitis-causing pathogens, and in **Chapter 4** we investigated if these typical characteristics of each pathogen could be used to find more effective indicators of CM. We defined several patterns of peaks in SCC, and tested their sensitivity and specificity, and estimated their associations with occurrence of pathogen-specific cases of CM. The definitions of the patterns of peaks in SCC were based on three or five consecutive test-day records. The SCC patterns differentiated between a short or longer period of increased SCC, and also between lactations with and without recovery. Presence of SCC patterns in a lactation predicted occurrence of overall CM in that lactation equally or even more accurate than presence of average lactation values of SCC of more than 200,000 cells/ml. Lactations with CM and with presence of the no recovery pattern had high probabilities for clinical *Staph. aureus* or CNS mastitis, but low probabilities for *E. coli* or culture-negative CM. The opposite was true for lactations with presence of the quick recovery pattern. *Streptococcus dysgalactiae* was not strongly associated with any of the defined patterns of peaks in SCC, and no single unambiguous pattern was found for *Strep. uberis*. Presence of patterns of peaks in SCC can be partly explained by pathogen-specific mammary gland pathogenesis, since pathogens do have typical characteristics.

Now that we knew that the pattern of peaks in SCC could distinguish between mastitis-causing pathogens, we investigated the use of patterns of peaks in SCC in genetic selection

and for udder health management to decrease the incidence of pathogen-specific CM. Genetic parameters for pathogen-specific CM, lactation-average SCC, and patterns of peaks in SCC were estimated in **Chapter 5**. Heritabilities for patterns of peaks in SCC ranged from 0.01 to 0.06, and the heritability for lactation-average SCC was 0.08. Genetic correlations between pathogen-specific CM and patterns of peaks in SCC differed per pathogen, and generally, they were stronger than the correlations with lactation-average SCC. Genetic selection on less presence of peaks in SCC would, therefore, decrease the incidence of pathogen-specific CM more effectively than selection on lower lactation-average SCC.

In **Chapter 6** we determined associations between incidence rates of pathogen-specific CM and of patterns of peaks in SCC on herd level. Incidence rates of patterns of peaks in SCC varied among herds, and information on the mean incidence rates of patterns of peaks in SCC and of currently used SCC traits are indicative for the occurrence of cases of overall CM, and of cases of CM associated with either contagious (CONT_CM) or environmental (ENV_CM) pathogens. The 25% herds with the highest incidence rate for the quick recovery pattern had 2.5 times higher risk to be classified as one of the 25% herds with highest incidence rates for CM. To be classified as one of the 25% herds with highest incidence rates for ENV_CM and CONT_CM they had 2.1 and 1.2 times more chance, respectively. The herds in the worst quartile for the incidence rate of the no recovery pattern had 1.1, 0.9 and 1.8 times more chance to be classified in the worst quartile for the incidence rate of overall CM, ENV_CM and CONT_CM, respectively. Based on the mean incidence rates of SCC patterns in a herd, the necessity to introduce pathogen-specific mastitis control programs in that herd, consisting of adequate guidelines to control the predominant type of mastitis, could be determined. Effects of changes in the udder health management on the status of the udder health in a herd can be easily evaluated based the incidence rates of the SCC patterns.

Combined results from the preceding chapters are discussed in **Chapter 7**, with a focus on the practical implementations, and recommendations with respect to (1) selection strategies and index selection, and (2) pathogen-specific mastitis control programs are given. The main conclusion with respect to selection strategies is that genetic selection for lower lactation-average SCC causes a shift in the main mastitis-causing pathogen. Therefore, there is a growing importance for defining new traits for SCC, which are based on biological understanding of pathogens and of the immune system of the cow. An index including the slow and no recovery pattern, plus the lactation-average SCC (305d) was most accurate to decrease the incidence of overall, environmental and contagious CM, and most likely to decrease the prevalence of SCM as well. The quick and no recovery pattern are useful as basic tools for health management advises. Presenting the incidence of these two patterns as a rolling average on the forms of the milk recording is of additional value. Management advises can then be directed specifically on changing herd risk factors related to predominant type of mastitis in a herd.

Samenvatting

Mastitis betekent letterlijk “ontsteking van het melkklierweefsel”, en is één van de meest voorkomende ziekten bij melkkoeien in Nederland. Ongeveer een kwart van alle Nederlandse melkkoeien krijgt een ontsteking in de uier in een lactatie. Een lactatie is de periode dat de koe melk geeft; deze periode is ongeveer 340 dagen. Een ontsteking in de uier kan veroorzaakt worden door diverse bacteriën, waarbij de belangrijkste groepen de stafylokokken, de colibacteriën en de streptokokken zijn.

Er zijn twee verschijningsvormen van mastitis te onderscheiden; klinische en subklinische mastitis. Klinische mastitis wordt gekenmerkt door zichtbare veranderingen in de melk en in de uier, zoals de kleur en de klontering van de melk, of een zwelling van de uier. Subklinische mastitis is niet duidelijk zichtbaar, maar kan opgemerkt worden door een toename van het aantal cellen per milliliter melk. Het aantal cellen per milliliter melk wordt het celgetal genoemd. De cellen in de melk zijn zowel epitheelcellen als witte bloedcellen. De epitheelcellen zijn afkomstig van het melkklierweefsel, en de witte bloedcellen horen bij het natuurlijke afweersysteem van de koe. Er zitten altijd cellen in de melk, maar als de afweer van de koe geprikkeld wordt doordat een mastitisveroorzaker via het tepelkanaal in de uier komt, reageert de koe met een toestroom van witte bloedcellen, om zo de strijd aan te gaan met deze bacterie. Dit heeft een stijging van het celgetal in de melk tot gevolg. Als het afweersysteem de infectie de baas wordt, dan verdwijnt de bacterie en daarmee ook de klinische verschijnselen, en gaat het celgetal weer omlaag. Als het afweersysteem de infectie niet aan kan, resulteert dat vaak in subklinische mastitis. De bacterie blijft dan aanwezig en het celgetal is verhoogd. Een snelle, adequate reactie van het immuunsysteem van de koe op het binnendringen van een bacterie is belangrijk voor een goede uiergezondheid.

Een veehouder verkrijgt informatie over het celgetal van zijn koeien door middel van melkcontrole. Ongeveer één keer per maand wordt een melkcontrole uitgevoerd. Hierbij worden melkmonsters genomen van iedere koe en tevens wordt de melkproductie van de koe geregistreerd. De melkmonsters worden geanalyseerd, waarbij het vet- en eiwitpercentage van de melk wordt bepaald, maar ook het celgetal. Deze melkproductie-registratie wordt gedaan door het NRS te Arnhem. Gedurende een lactatie blijft het celgetal niet op een constant niveau, maar verloopt gemiddeld gesproken volgens een bepaald patroon. In het begin van de lactatie heeft de koe een hoog celgetal, waarna deze gedurende ongeveer twee maanden daalt en vervolgens langzaam stijgt tot aan het einde van de lactatie (zie figuur 3.1; blz. 52). Verschillen tussen koeien in het celgetalpatroon kunnen deels verklaard worden door de leeftijd, het afkalfseizoen en de erfelijke aanleg van de koe. Ook klinische en subklinische mastitis veroorzaken afwijkingen ten opzichte van het standaardpatroon van het celgetal.

Samenvatting

Dit proefschrift richt zich met name op klinische (en minder op subklinische) mastitis, omdat dit een ziekte is die grote economische verliezen veroorzaakt voor de Nederlandse melkveehouderij. De verliezen worden enerzijds veroorzaakt door verminderde opbrengsten doordat de melkproductie van een zieke koe lager is dan die van een gezonde koe. Anderzijds zijn de onkosten hoger omdat de behandeling van een zieke koe met antibiotica geld kost. Een koe die goed produceert en niet ziek is, is daarom vanuit economisch oogpunt belangrijk voor een veehouder. Het staat natuurlijk buiten kijf dat het voor het welzijn van de koe zelf ook bevorderlijk is dat ze niet ziek wordt. Ook voor de volksgezondheid is het belangrijk om het optreden van klinische mastitis te verminderen, vanwege het gevaar dat bacteriën, die schadelijk zijn voor de mens, resistent worden tegen antibiotica. Melkveehouders proberen het optreden van klinische mastitis te verminderen door de bedrijfsvoering te verbeteren (melkmachine, huisvesting), en door middel van fokkerij.

In dit proefschrift staat de vraag centraal of we patronen in het verloop van het celgetal kunnen definiëren die aangeven met welke mastitisveroorzaker we waarschijnlijk te maken hebben. Hiervoor zijn koeien gegroepeerd per bedrijf, of op basis van hun vader. Deze informatie kan dan gebruikt worden in de bedrijfsvoering en in de fokkerij.

Als een koe klinische mastitis krijgt in een lactatie, dan veroorzaakt dit afwijkingen ten opzichte van het normale celgetalpatroon. Zoals eerder gezegd, zijn er drie belangrijke groepen van bacteriën die klinische mastitis kunnen veroorzaken. Uit onderzoek van de Gezondheidsdienst voor Dieren is gebleken dat de frequentie waarmee mastitisveroorzakers hun werk doen, is gewijzigd in de afgelopen decennia (zie tabel 7.1; blz. 132). In de jaren ’50 was *Streptococcus agalactiae* de belangrijkste mastitisveroorzaker en momenteel zijn *Staphylococcus aureus*, *Escherichia coli* en *Streptococcus uberis* de belangrijkste mastitisveroorzakers in Nederland. Iedere groep heeft specifieke eigenschappen. De stafylokokken bezitten de eigenschap om zich in het uierweefsel te nestelen. Er zijn daardoor meestal niet dermate veel stafylokokken aanwezig dat de koe ook daadwerkelijk ziek wordt, maar het immuunsysteem van de koe wordt wel continu geprikkeld, omdat zich een lichaamsvreemde stof in de uier bevindt. Een gevolg hiervan is dat het celgetal van de koe gedurende lange tijd verhoogd is. Dit in tegenstelling tot de colibacteriën die bekend staan om hun acute verschijningsvorm. Men zegt wel: “*E. coli* komt en gaat”, en zo is het ook. Maar intussen zorgt het wel voor een extreem zieke koe, en soms overleeft de koe een *E. coli* infectie niet eens. Het celgetal vertoont daarom vaak alleen een kortstondige piek, en verder nauwelijks een verhoogd celgetal. De streptokokken lijken er een beetje tussenin te zitten; ze verschijnen niet zo acuut als de colibacteriën, maar ze blijven ook niet zo lang sluimeren als de stafylokokken (zie figuur 3.2; blz. 58).

Om informatie te verkrijgen over het voorkomen van deze verschillende piekpatronen, hebben we drie patronen gedefinieerd op basis van de geregistreerde celgetallen bij opeenvolgende melkcontroles. Het eerste patroon is een korte piek, waarbij op drie achtereenvolgende melkcontroles een laag, hoog en laag celgetal geregistreerd moet zijn (zie figuur 4.1; blz. 72). We hebben aangetoond dat deze piek voornamelijk geassocieerd kan worden met de colibacteriën. Het tweede patroon is een langzamere piek in het celgetal, en is gedefinieerd als zijnde laag, hoger, hoog, lager, laag. Dit patroon werd vooral gevonden in lactaties van díe koeien die klinische mastitis kregen doordat een streptokok of een stafylokok binnendrong in de uier. Het derde patroon omvat een langdurige verhoging van het celgetal, met achtereenvolgens een laag, hoog, hoog, hoog, hoog celgetal. Deze langdurige celgetalverhoging was sterk geassocieerd met de stafylokokken.

Een stap verder is om te onderzoeken of de piekpatronen die wij gedefinieerd hebben ook gebruikt kunnen worden voor de fokkerij. Tot voor kort was het lactatiegemiddelde van het celgetal de belangrijkste informatiebron voor de uiergezondheid. Oftewel, er werd dus gekeken naar het gemiddelde van de geregistreerde celgetallen op alle afzonderlijke melkcontroles gedurende de lactatie. De gedachte hierachter was dat een koe met een laag gemiddeld celgetal een goede uiergezondheid heeft, en dus werden die koeien geselecteerd die lage gemiddelden hadden. Het nadeel van een gemiddelde is dat je geen onderscheid maakt tussen een koe met een langdurige lichte verhoging van het celgetal, en een koe met één enorme piek in het celgetal en verder lage celgetallen. Beide koeien zouden hetzelfde gemiddelde kunnen hebben, maar de getoonde celgetalpatronen duiden echter op verschillende prikkelingen van het immuunsysteem van de koe.

We hebben onderzocht of we met het selecteren op minder piekpatronen in het celgetal de uiergezondheid van de koe meer konden verbeteren dan door te selecteren op een lager lactatiegemiddelde van het celgetal. Dat bleek inderdaad zo te zijn, maar alleen door te kijken naar piekpatronen in het algemeen, en niet zozeer door te kijken naar de afzonderlijke piekpatronen. Aan de gevonden relaties tussen de piekpatronen in het celgetal en de mastitisveroorzakers bleek dus niet de genetische aanleg van de koe ten grondslag te liggen. Aan de andere kant, koeien met überhaupt veel pieken in het celgetal zijn van nature vatbaarder voor klinische mastitis, dan koeien met weinig pieken in het celgetal (erfelijke relatie = 0,90). Deze relatie was sterker dan de relatie tussen het lactatiegemiddelde van het celgetal en de mastitisveroorzakers (erfelijke relatie = 0,39). Dit impliceert dat er meer vooruitgang geboekt kan worden door te selecteren tegen het aantal pieken in het celgetal, dan op een lager lactatiegemiddelde.

Samenvatting

Informatie over het voorkomen van piekpatronen bij koeien op een bedrijf is bruikbaar voor de bedrijfsvoering. Melkveebedrijven waar de koeien veelal korte pieken in het celgetal hebben, hebben meer problemen met colibacteriën dan met stafylokokken als mastitis-veroorzakers. Het tegenovergestelde geldt voor bedrijven waar het celgetal van de koeien vaker langdurig verhoogd is. Met de informatie over de piekpatronen in zijn achterhoofd zou de veehouder de bedrijfsvoering aan kunnen passen richting één van de bacteriegroepen. Voor colibacteriën en sommige streptokokken (zoals *Streptococcus uberis*) zullen die aanpassingen vooral gezocht moeten worden in de omgeving van de koe. Daarbij valt te denken aan droge ligboxen met schoon strooisel, en schone uierdoeken. Voor de stafylokokken en andere streptokokken (zoals *Streptococcus dysgalactiae*) zullen de aanpassingen vooral gezocht moeten worden in het voorkómen van een besmetting van de ene koe door een andere koe. Dat kan door besmette koeien op te sporen aan de hand van de uitslag van een melkcontrole, en dan te beslissen over behandeling of afvoer van de koe. Besmetting met iedere mastitisveroorzaker kan makkelijk plaatsvinden in de melkstal doordat melk van een besmette koe achterblijft in het melkstel, en dan zo de volgende koe die met dit melkstel wordt gemolken kan besmetten. Een goed functionerende melkmachine is daarom cruciaal om besmettingen te beperken.

De conclusie van dit proefschrift is dat piekpatronen in het celgetal informatiever zijn voor de status van de uiergezondheid van een koe, dan het lactatiegemiddelde van het celgetal. De piekpatronen kunnen gebruikt worden voor de fokkerij, aangezien koeien met weinig pieken in het celgetal minder vatbaar zijn voor mastitis, dan koeien met veel pieken. Voor de fokkerij kijken we dus niet zozeer naar de afzonderlijke piekpatronen, maar meer naar het voorkomen van überhaupt een piek in het celgetal. De piekpatronen gelden ook als informatiebron voor de bedrijfsvoering, aangezien onderscheid gemaakt kan worden tussen bedrijven met voornamelijk korte pieken in het celgetal, en bedrijven met vooral langdurig verhoogde celgetallen. Dit kan weer gerelateerd worden aan welke bacterie het belangrijkste is als mastitisveroorzaker op een bedrijf.

Tot slot

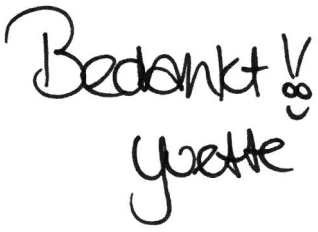
Nawoord

Ik ben dit proefschrift begonnen met een schilderij, en zo wil ik het ook graag afsluiten (vrij naar "Het Schilderij" – Guus Meeuwis & Vagant). Het leven is soms net een schilderij…

Je ouders zijn de eerste die gaan schilderen
Voorzichtig rood en later geel en groen
Omdat als je leerling bent geworden
Tot hun spijt steeds meer zelf wil gaan doen

En dan gaan zich er anderen mee bemoeien
Je vrienden, ze mengen in het café
Van alles door elkaar, het is een zooitje
Maar de mooiste neem je heel je leven mee
En degenen die hun streken achterlieten
Veel te veel en veel te blauw ook bovendien
Ze zijn nu lucht, maar dat was ooit wel anders
Een zee van tranen en geen horizon te zien
Het leven is soms net een schilderij
De tijd verstrijkt er steeds een kleurtje bij

Vele vrienden, familieleden, begeleiders, docenten, collega's en eigenlijk iedereen die interesse heeft getoond, hebben door de jaren heen een kleurtje bijgedragen aan 'mijn schilderij'; teveel mensen om hier bij naam te noemen, dus daarom bedank ik niemand in het bijzonder, maar iedereen in één keer.



Curriculum Vitae

"Mijn naam is Haas!"; Yvette de Haas. Ik ben op 3 juli 1974 in Nijmegen geboren. In mei 1992 heb ik mijn VWO-diploma gehaald aan de Stedelijke Scholengemeenschap Nijmegen, waarna ik in 's-Hertogenbosch aan de Hogere Agrarische School ben gaan studeren. Daar ben ik in augustus 1996 afgestudeerd in de studierichting Veehouderij, met als specialisatie Rundveehouderij. In datzelfde jaar ben ik begonnen met het doorstroomprogramma van de studie Zoötechniek aan de toenmalige Landbouwuniversiteit te Wageningen. Hiervoor heb ik een studiebeurs van de Rommert D. Politiek Stichting gekregen. In september 1999 heb ik deze studie afgerond in de oriëntatie Veefokkerij, met lof en scriptieprijs voor het afstudeervak dat ik in aan de Landbouwuniversiteit van Uppsala, Zweden, heb volbracht. Een tweede afstudeervak in de oriëntatie Veefokkerij heb ik bij Ross Breeders in Edinburg, Schotland, uitgevoerd. In september 1999 ben ik aangesteld als Assistent in Opleiding (AIO) bij de leerstoelgroep Fokkerij en Genetica van Wageningen Universiteit, en was gedetacheerd bij de divisie Dier en Omgeving van het Instituut voor de Dierhouderij en Diergezondheid (ID-Lelystad; tegenwoordig Animal Sciences Group). Ik verrichtte daar het onderzoek dat in dit proefschrift is beschreven.

List of Publications

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Aantekeningen

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