Epidemiological Studies of Risk Factors for Bovine Mastitis

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Abstract

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This thesis describes some factors associated with mastitis in Swedish dairy herds, and with udder health of first-parity dairy cows. These factors were investigated in four observational studies including a total of 343 dairy herds and 3315 first-parity cows throughout Sweden. The herds were visited and the farmers interviewed about management, housing, feed, feeding, and milking. The quality of feed, as well as presence of udder pathogens at a case of clinical mastitis, was investigated in two of the studies. Blood samples were taken from first-parity cows in the period two weeks before calving to six weeks after calving for analysis of metabolites and immunological parameters in one of the studies. Udder health measurements used as outcomes in the studies were being a herd with a low or high incidence rate of veterinary treatments of clinical mastitis (CM), being a first-parity cow veterinary treated for CM in the period -7 to 30 days after calving, the within herd number of first-parity cows veterinary treated for CM in the period -10 to 60 days after calving, being a first-parity cow with somatic cell count (SCC) $\geq$ 200,000 cells/mL at first test-milking, the within herd number of first-parity cows with SCC $\geq$ 200,000 cells/mL at first test-milking, and as SCC of first-parity cows at first test-milking. Associations between the registered variables and udder health were statistically analyzed using multivariable linear, logistic and Poisson regression models. Results showed that farmer attitude toward treatment of a case of mastitis differed, some contacted a veterinarian at very mild signs of mastitis, while others waited until the cows showed more clinical signs. Moreover, several feed and feeding variables, and hygienic parameters, especially around calving, were found associated with udder health. Furthermore, the results showed that cows of the Swedish Red breed and herds with the majority of cows of this breed, on average have a more beneficial udder health status than cows, or herds with the majority of cows, of the Swedish Holstein breed. Several metabolites and immunologic parameters were associated with SCC at first test-milking, and most of these parameters varied significantly between breed and between first-parity cows of different age at first calving.

Keywords: mastitis, dairy cows, first-parity cows, management, feeding, milking, housing, metabolites, immune parameters, breed

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Epidemiology is like bowling, although you try to do everything the same way each time and aim at the middle, you are as likely to get a result skewed (less or more) to the right or left, and sometimes you end up in the gutter!

Contents

Appendix

Papers I-IV

The present thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. Nyman, A.-K., Ekman, T., Emanuelson, U., Gustafsson, A.H., Holtenius, K., Persson Waller, K., and C. Hallén Sandgren. 2007. Risk factors associated with the incidence of veterinary-treated clinical mastitis in Swedish dairy herds with a high milk yield and a low prevalence of subclinical mastitis. *Preventive Veterinary Medicine* 78:142-160.

II. Svensson, C., Nyman, A.-K., Persson Waller, K., and U. Emanuelson. 2006. Effects of housing, management, and health of dairy heifers on first-lactation udder health in southwest Sweden. *Journal of Dairy Science* 89:1990-1999.

III. Nyman, A.-K., Emanuelson, U., Gustafsson, A.H., and K. Persson Waller. Management routines associated with udder health of first-parity dairy cows in early lactation (s*ubmitted*).

IV. Nyman, A.-K., Emanuelson, U., Holtenius, K., Ingvartsen, K. L., Larsen, T, and K. Persson Waller. Metabolites and immune parameters associated with somatic cell counts of primiparous dairy cows (*manuscript*).

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Abbreviations

BHBA	β-OH-butyrate
BMSCC	bulk milk somatic cell count
CM	clinical mastitis
CR	central range
DM	dry matter
ECM	energy corrected milk
HI	high incidence
HP	hard pressed
IRVTCM	incidence rate of veterinary treatments of clinical mastitis
LO	low incidence
LSM	least square mean
NEB	negative energy balance
NEFA	nonesterified fatty acids
PMR	partial mixed ration
SADSR	Swedish animal disease recording scheme
SCC	somatic cell count
Se	selenium
SH	Swedish Holstein breed
SOMRS	Swedish official milk recording scheme
SR/SRB	Swedish Red breed
TMR	total mixed ration
UDS	udder disease score
VTCM	veterinary treated for clinical mastitis

General introduction

Dairy farming in Sweden

In Sweden there are approximately 380,000 dairy cows in about 8,000 herds. During the last decade the number of dairy herds has been reduced by half, while the number of cows per herd has increased by two thirds (on average 48 cows/herd in 2006). In the same period milk production has increased with approximately 15% to 9,300 kg ECM/cow. There are two main dairy breeds in Sweden, the Swedish Red (SR) and the Swedish Holstein (SH) breed, constituting 44% and 50% of the Swedish dairy cow population, respectively. A Swedish cow usually calves for the first time at 28 months of age, and is culled after 2.7 lactations. On average, 37% of the cows in a herd are first-parity cows.

The majority of dairy cows are housed in tie stalls, and only about 16% of the cows are housed in free stalls. A dairy cow in Sweden is traditionally fed a diet based on grass- or grass/clover-silage, grain (most common barley and oats) and industrially-processed concentrates. However, it has become more common with diets also including corn silage, and hard pressed (HP) sugar-beet pulp, but this occurs primarily in the south of Sweden.

Through the Swedish official milk-recording scheme (SOMRS; (Olsson *et al.*, 2001) and the Swedish animal disease recording system (SADRS; (Emanuelson et al., 1998; Olsson *et al.*, 2001), information is available on milk production and cow health for approximately 78% of the Swedish dairy herds and 86% of the Swedish dairy cows. In Sweden, only veterinarians are allowed to start an antibiotic treatment of an animal and every treatment should be reported to the SADRS, which is administered by the Swedish Board of Agriculture and linked to the SOMRS.

Mastitis

Mastitis is the most common disease of dairy cows representing 47% of all veterinary treated diseases in Sweden (Swedish Dairy Association, 2006). Mastitis causes a reduced milk production, not only at the occurrence of the mastitis but throughout the rest of the lactation (Gröhn *et al.*, 2006; Hagnestam, Emanuelson & Berglund, 2007), increases the risk of new cases of mastitis (Edinger *et al.*, 1999; van Dorp *et al.*, 1999), and increases the risk of culling (Dohoo & Martin, 1984b; Schneider *et al.*, 2007). The welfare of the cow is negatively influenced by mastitis as it can induce pain and even cause death. Consequences for the farmer are economic losses mainly due to reduced milk production and increased culling. Mastitis is not just an issue for the cow and farmer, but also for the consumers. Consumers expect that milk comes from healthy animals, and the quality of milk is negatively influenced by mastitis.

Predisposing factors in the management and environment cause mastitis by negatively influencing the local and systemic barriers and defence of the cow, and/or by increasing exposure of the udder to micro-organisms. A case of mastitis can be classified as either subclinical or clinical, depending on how severe the infection becomes, which in turn depends on how resistant the host (the cow) is to disease (IDF, 1987; Hillerton, 1996). Most commonly the micro-organisms isolated at mastitis are bacteria. These are usually separated into contagious or environmental bacteria. The mammary gland and/or teat skin are the main reservoirs for contagious bacteria, while the environment, *e.g.* bedding, is the main reservoir for environmental bacteria. The most common contagious bacteria mentioned in the literature are *Staphylococcus (S.) aureus, Streptococcus (Str.) agalactiae,* and *Str. dysgalactiae*. The most common environmental bacteria are coliforms (*e.g. Escherichia (E.) coli and Klebsiella* spp.*), Str. uberis,* and *Enterococci* spp. Other bacteria of importance, are coagulase-negative staphylococci (CNS) and *Arcanobacterium (A.) pyogenes.* Contagious bacteria are most often spread between quarters and cows at milking (by the milking units or the hands of the milker), but may also be spread by intersucking between calves/heifers and by flies. Environmental bacteria are transferred to the teats from the environment between milkings (*e.g.* when the cow lies down), but could also, to a lesser extent, be transmitted between udder quarters and cows at milking.

Subclinical mastitis

Subclinical mastitis is characterized by changes in milk composition *e.g.* somatic cell count (SCC; leukocytes and epithelial cells), but also by changes in milk pH and ion concentration, without clinical signs of inflammation (Guidry, 2007). In the healthy lactating mammary gland, the milk SCC is often < 100,000 cells/mL of milk, while the SCC can increase to > 1,000,000 cells/mL of milk during subclinical mastitis. The major factor affecting the SCC at the herd and individual level is the presence of intramammary infections (Radostits, 2007). In Sweden, the most common pathogens causing subclinical mastitis are *S. aureus*, CNS, and *Str. dysgalactiae* (Swedish Dairy Association, 2006).

The recommendation in Sweden is to not treat cows with high SCC with antimicrobials during lactation, but to use dry-cow treatment at drying off, and to cull chronic cases. It is also important to milk high SCC cows last to reduce the risk of transmitting bacteria to non-infected cows. However, the dairies in Sweden give economical penalties to milk producers delivering high SCC milk and promote producers delivering low SCC milk (since the SCC influences the milk quality), which might induce the farmer to use antimicrobial treatment of cows with high SCC during lactation.

Clinical mastitis

Clinical mastitis is characterized by visual clots or discolorations of the milk, often in combination with tender and swollen udder, sometimes in combination with fever, loss of appetite *etc*. In Sweden, the most common pathogens isolated at clinical cases of mastitis are *S. aureus, E. coli, Str. dysgalactiae* and *Str. uberis* (Bengtsson *et al.*, 2005).

Clinical mastitis is often treated with antimicrobials. In Sweden, penicillin is used in most cases, and if needed, in combination with non-antimicrobial treatment (*e.g.* non-steroid anti-inflammatory drugs, fluid therapy). Frequent milking and massage of the udder is also recommended. Records of veterinary treated clinical cases of mastitis (VTCM) are available from the SADRS since the early 1970's on a national and regional level.

Use of antimicrobials and development of antimicrobial resistance

Approximately 70% of the antimicrobials used in dairy production is for treatment of clinical mastitis (Waldner, 2002). According to SVARM (2001) the use of antimicrobials have increased by 37% from 1990 to 2000. The use of antimicrobials have, overtime, increased the number of antimicrobial-resistant microbes globally, and any use of antimicrobial agents will to some extent benefit the development of resistant strains (Williams, 2000). The occurrence of antimicrobial resistance in microbes makes it more difficult to treat individual animals. Unnecessary or inappropriate usage (wrong dose, drug or duration) contributes the most to the increase in antimicrobial resistance without improving the outcome of treatment (Williams, 2000). In Sweden, antimicrobial resistance in microbes, isolated from production animals, is rare (SVARM, 2006) and the restricted use of antimicrobials in animal production could be one factor contributing to this advantageous situation. Moreover, the first-hand choice of antimicrobials for treatment of mastitis in Sweden is penicillin (since gram-positive bacteria are the most common finding at a case of mastitis) which is an antimicrobial with low risk of inducing resistance. As reported by SVARM (2002) only 7% of *S. aureus* isolated at cases of CM were resistant against penicillin (ß-lactamase producing). This can be compared to Denmark (2003), Finland (2001), and Island (2006) where 23%, 52%, and 68% of *S. aureus* isolates from milk samples from cases of CM, respectively, were resistant against penicillin (Landin, 2007, personal communication). Preventive health measures and reduction of unnecessary antimicrobial treatments will aid in keeping the beneficial situation, with low levels of antimicrobial resistance in microbes in Sweden.

Epidemiology of mastitis

Several epidemiological studies have been performed investigating factors associated with, and influencing the risk of, mastitis on udder quarter, cow and herd level. Those studies have found factors associated with udder health in the areas of milk production and cow characteristics (including *e.g.* yield, breed and bulk milk SCC (BMSCC)), health and preventive health measures, housing and cleanliness, milking and milking hygiene, and feeding (Bakken, 1982; Schukken *et al.*, 1990; Elbers *et al.*, 1998; Waage, Sviland & Odegaard, 1998; Barkema *et al.*, 1998a; Barkema *et al.*, 1999; Peeler *et al.*, 2000; Waage *et al.*, 2001; De Vliegher *et al.*, 2004b; Barnouin *et al.*, 2005; O'Reilly *et al.*, 2006; Valde *et al.*, 2007; Compton *et al.*, 2007). Some factors most commonly found to be associated with udder health in these studies were increased number of cows leaking milk, high milk production, very low BMSCC, breed, older age at calving, teat injuries, hygiene of calving area, and use of post-milking teat disinfection.

In Sweden, epidemiological studies of mastitis have showed associations between udder health and teat injuries, other diseases (*e.g.* retained placenta), housing system, milk production, breed, herd size, milking technique, and management (Bendixen *et al.*, 1988; Emanuelson, Oltenacu & Grohn, 1993; Oltenacu & Ekesbo, 1994; Ekman, 1998). However, only Ekman (1998) looked at aspects of management, housing, milking, feed and feeding factors in the whole herd (including calf, heifer, dry-cow and lactating cow management), and associations with herd SCC. A similar study investigating factors associated with the incidence of VTCM in dairy herds has not been performed in Sweden. Moreover, epidemiological studies of mastitis performed in other countries may differ somewhat in management, housing, milking, feed and feeding compared to the Swedish situation making it difficult to apply findings from those studies to Swedish dairy herds. The most common housing system for dairy cows in Sweden (as in most other Scandinavian countries) is still tie stalls, while free stalls are more common in other countries in Europe, and in USA, Canada, Australia and New Zealand from where epidemiological studies of mastitis most commonly have been reported. However, the number of free stalls in Sweden increases with increasing herd size. The strict Swedish animal-welfare regulations on animal housing make housing systems of the same type (tie stalls with short stalls, tie stalls with long stalls *etc.*) rather similar, and perhaps not completely comparable to housing systems in other countries.

To our knowledge, the association between feeding and udder health has not been investigated extensively in any country. In contrast to the diets used in Sweden (mostly grass-silage in combination with grain and industrially-processed concentrates), corn silage and soy, and/or a more pasture based system, is used to a much greater extent in many other countries. Moreover, the SR-breed is a unique Swedish breed, making it difficult to compare studies performed mainly on cows of the Holstein breed in other countries.

Another concern, making epidemiological studies of mastitis in Sweden important, is that while the BMSCC has improved during the last decades, though actually increasing slightly during the last four years, the incidence of VTCM has been approximately the same (SHS, 1982; Swedish Dairy Association, 2003; Swedish Dairy Association, 2006). This indicates that the efforts in improving the BMSCC have not had much influence on the incidence of VTCM. Finding factors of importance for udder health, measured both as SCC and CM, and implementing those factors would improve Swedish dairy farming, be advantageous for the dairies, and enhance the consumer’s trust.

Important changes in metabolism and associations with immune suppression in the period around calving and in early lactation

The period around calving and in early lactation is associated with immune suppression. The physiological and metabolic changes during calving and the onset of lactation, as well as external factors in the environment and management, can contribute to this immune suppression (*e.g.* reviewed by Mallard *et al.* (1998) and Persson Waller (2000)). It has been shown that some of the changes in this period directly associated with calving (*e.g.* increases in plasma cortisol and estrogen concentrations), or exclusively due to the onset of lactation, have negative effects on various immune functions (Goff, Kimura & Horst, 2002; Kimura *et al.*, 2002). Some cows have difficulties in coping with these changes/demands, and metabolic disorders and infections are common in this period (Ingvartsen, 2006).

As a response to the large nutrient demand during the periparturient period and in early lactation, nutrients are mobilized from tissue reserves. The metabolic changes can be measured as marked alterations in blood concentrations of a large number of hormones and metabolites (Ingvartsen & Friggens, 2005). The large need of glucose for milk lactose production causes a decrease in blood glucose concentration. In addition, insulin concentrations also decrease in this period. The lower insulin concentration shifts the balance between lipogenesis and lipolysis causing mobilization of fat from the adipose tissue resulting in a rise in nonesterified fatty acids (NEFA) in plasma, while the increased ketogenesis in hepatic tissue increases the level of ketone bodies (*eg.* β-OH-butyrate (BHBA)) in blood. Elevated NEFA and BHBA concentrations, and decreased glucose and insulin concentrations, are indications of negative energy balance (NEB; Rukkwamsuk, Kruip & Wensing, 1999; van Knegsel *et al.,* 2005). Impaired immune functions and udder health of dairy cows have been shown to be associated with both high levels of NEFA and BHBA (reviewed *e.g.* by Ingvartsen, Dewhurst & Friggens (2003) and Burvenich *et al.* (2007)). The exact relationship between different metabolites and immune parameters are not known, and further investigations are advocated.

Cholesterol is one of the metabolites involved in the process of transforming triglycerides to very low density lipoproteins in order to transport triglycerides from the liver to different organs and tissues. Low levels of cholesterol can increase the risk of fatty liver (impairment of the liver function) due to reduced export of triglycerides from the liver (Holtenius, 1989). At calving, cholesterol levels are usually low, and they increase slowly after calving (Drackley *et al.*, 1992; Weiss *et al.*, 1997; Cavestany *et al.*, 2005). Cholesterol levels, both low and high, have been shown to be associated with different metabolic disorders (Itoh *et al.*, 1998).

Urea levels are associated with protein metabolism, and high levels can be an indication of high dietary crude protein levels fed to the cow, but can also be an indicator of mobilization of body proteins. However, urea levels usually decrease as the animal approaches calving (Greenfield *et al.*, 2000; Dorshorst & Grummer, 2002; Odensten *et al.*, 2007).

Some nutrients, *e.g.* vitamin E and selenium (Se), are essential for vital functions of cows and other mammals. As the cow approaches calving blood concentrations of vitamin E usually decrease (Goff & Stabel, 1990; Weiss *et al.*, 1990; Meglia *et al.*, 2001), while concentrations of Se have been shown both to increase (Weiss *et al.*, 1990; Meglia *et al.*, 2001) and decrease (Miller *et al.*, 1995). Supplementation with vitamin E and Se has been shown to be associated with enhanced immune functions of dairy cows (Hogan *et al.*, 1992; Cebra *et al.*, 2003), and some have found beneficial effects of vitamin E and Se supplementation on udder health (Smith *et al.*, 1984; Weiss *et al.*, 1990). Investigations on associations between vitamin E, and Se levels, and udder health in Sweden can contribute important understanding of the need of supplementary feeding of these nutrients.

First-parity cows and udder health

In Sweden, most first-parity cows calve and start to lactate at about 2-2.5 years of age. To pay for the costs of rearing it is very important that the first-parity cow is healthy, and able to produce good quality milk, which also will enhance longevity. Unfortunately, first-parity cows have been shown to have as high, or higher, incidence of udder disorders in early lactation as older cows (Miltenburg *et al.*, 1996; Barkema *et al.*, 1998b; Valde *et al.*, 2004). This can be detrimental to her future life due to reduced milk production (Gröhn *et al.*, 2006; Hagnestam, Emanuelson & Berglund, 2007), increased risk of new cases of mastitis (Edinger *et al.*, 1999; van Dorp *et al.*, 1999), increased SCC throughout the lactation (De Vliegher *et al.*, 2004a), and increased risk of culling (Dohoo & Martin, 1984b; Waage *et al.*, 2000; De Vliegher *et al.*, 2005; Compton *et al.*, 2007a; Schneider *et al.*, 2007). The physiological changes, as well as changes in management and environment, around parturition are stressful, and likely more stressful for first-parity cows than for older cows. Therefore, research on how to keep the first-parity cow healthy and well-producing is of importance, and has not been extensively performed in Sweden. There have been studies of factors associated with the udder health of first-parity cows in other countries. It was found that herd milk yield, BMSCC, overall incidence of CM in the herd, age at calving, breed, udder edema, milk leakage at calving, introduction to the lactating cow housing before calving, hygiene of the calving area, amount of corn silage given, amount of concentrates given in transition diets, and serum NEFA concentrations post-calving were associated with udder health (Waage, Sviland & Odegaard, 1998; Bareille *et al.*, 2000; Waage *et al.*, 2001; De Vliegher *et al.*, 2004b; Compton *et al.*, 2007b).

Aims of the study

The general aim of this thesis was to gain further knowledge of the epidemiology of mastitis in Swedish dairy herds presumed to represent dairy herds of tomorrow. Knowledge of factors influencing udder health will hopefully aid farmers to reduce the incidence of mastitis in their herd, and to reduce excessive use of antimicrobials.

More specifically the aims were:

- To investigate differences in management, feeding, housing and milking practices between herds with a high or low incidence of VTCM.
- To describe the incidence of subclinical and clinical mastitis of first-parity cows in early lactation, and to investigate factors in management, feeding, housing, and milking practices from birth to calving influencing these incidences.
- To investigate if different factors in the areas of management, feeding, housing, and milking of first-parity cows are associated with different udder health measures.
- To investigate the influence of management, feeding and housing in the period around calving on udder health of first-parity cows.
- To investigate if metabolic and immunological status around calving are associated with udder health of first-parity cows.

Material and Methods

In this section, the material and methods applied in the studies of this thesis are summarized. A more detailed description is given in each paper (I-IV).

Study populations

Herds eligible for selection in all studies had to be enrolled in the SOMRS and the SADRS. The herds participating in the studies reported in papers I, III and IV were located throughout Sweden with the majority of farms situated in the southern half of Sweden. All herds included in paper II were located in the county of Skaraborg in the southwest of Sweden. A summary of the selection criteria is shown in Table 1.

Table 1. *Summary of selection criteria for herds participating in four different studies of factors associated with udder health of dairy cows in Sweden (1997-2006)*

Study	Herd size, cows/year	Milk production, kg of milk	Proportion of cows with UDS[a] 0 to 2	BMSCC, cells/mL	Housing
I	> 20	> 8,000	> 69	-	-
II	28-94	-	-	-	individual or group pens for calves
III and IV	≥ 80	> 8,900	-	≤ 192,000	free stalls for lactating cows

[a]Animals with UDS 0 to 2 have a probability of 0–29% that one or more udder quarters are infected. The score corresponds approximately to having a SCC of < 131,000 cells/mL on three consecutive test-milkings.

In the study presented in paper I the herds had to have a herd size > 20 cows, and belong to the top third regarding milk production, the lowest quarter regarding prevalence of subclinical mastitis (measured as proportion of cows with udder disease score (UDS) 0 to 2), and the lowest quarter (LO-herds) or highest fifth (HI-herds) concerning IRVTCM during the years of selection. UDS is a measure of the udder health of an individual cow, based upon three consecutive months of test-milking results of the cow SCC (Brolund, 1990), and expresses the probability that a cow has mastitis in one or more udder quarters at a given test-milking. UDS 0 to 2 imply a probability of infection of 0-29%, and correspond approximately to having a SCC of < 131,000 cells/ml on three subsequent test milkings. Herds were selected at two occasions: in 1997 based on data from September 1994 to August 1997, and in 1998, based on data from September 1995 to August 1998. A total of 158 herds, 79 LO-herds and 79 HI-herds, agreed to participate in the study. The study period was from September 1 to August 31 during the year of participation (1998 or 1999).

The farms selected in study II had to have a herd size of 28-94 cows and have their young calves in individual or group pens. A total of 107 herds participated, including 2,126 heifers. Heifers born in 1998 were monitored from birth to first calving (or culling). The majority of heifers calved in 2000-2001.

The herds in study III had to house their lactating cows in free stalls, have ≥ 80 cows, an annual milk production above the Swedish average, and a BMSCC below the Swedish average. A total of 78 herds agreed to participate. Of those, 20 herds with the highest number of predicted first-parity cows calving were asked to participate in the study presented in paper IV. The study period was from October 1, 2005 to March 31, 2006, but only heifers calving between October 15, 2005 and January 15, 2006 were monitored.

Study designs

The study reported in paper I was an ecologic retrospective case-control study investigating factors associated with udder health at herd level, while the studies in papers II and III were prospective longitudinal and single cohort studies, respectively, looking at both herd and individual factors associated with udder health of first-party cows. The study in paper IV was a prospective single cohort study looking at individual blood parameters and associations with udder health of first-parity cows at first test-milking.

Udder health measures used

Udder health was measured in different ways in the studies. In paper I udder health was measured as the IRVTCM and the herds were classified as case (HI-herds) if they belonged to the highest fifth, or control (LO-herd) if they belonged to the lowest quarter concerning IRVTCM in Swedish herds enrolled in the SOMRS and SADRS during the years of selection. The IRVTCM was calculated as (total number of cases/total number of cow-days in herd)*365*100, thus representing the number of cases per 100 cow-years. In paper II udder health was measured at the individual level in two different ways; being VTCM in the period -7 to 30 days after calving, and having a SCC ≥ 200,000 cells/mL at first-test milking. The udder health measures used in paper III were slight modifications of those used in paper II *i.e.* the within-herd number of first-parity cows VTCM in the period -10 to 60 days after calving, the within-herd number of first-parity cows with SCC ≥ 200,000 cells/mL at first-test milking, and the individual SCC at first test-milking. The individual SCC at first test-milking was also used as measure of udder health in paper IV.

Collection of data

Herd data from the SOMRS and the SADRS including veterinary-treated cases of diseases, udder disease scores, fertility data, milk yield, BMSCC, parity distribution, and breed composition were collected for all studies. Herd data was collected for the year of participation. Information about herd milk quality (*e.g.* bacterial counts, spores) and barn water quality (*e.g.* bacterial count, pH) was obtained from the dairies for the year of participation for the study presented in paper I. Individual cow data was also collected from the SOMRS and SADRS for the studies reported in papers II-IV. The individual data included calving date, difficulties at calving, age at calving, veterinary-treated diseases, breed, production (milk yield, fat, protein, milk urea and cow composite SCC at monthly test-milkings), and data on culling. Individual data was collected from calving to 305 days of lactation, or culling, for heifers in study II, while data for heifers participating in studies in papers III-IV were collected from two weeks before calving to two months after calving.

Questionnaires

Questionnaires were used and the farmers were interviewed at the farm in the studies reported in papers I-III. The questionnaire used at the interview in study I was based on a questionnaire used in a pilot-study with 20 LO-herds and 20 HI-herds (not published), and the questionnaire used at the interview in study III was based on the questionnaires used in studies I and II. In addition, the farmers were asked to fill in additional questionnaires during the study periods in studies I-III. These questionnaires were collected at the farm or by mail.

Observation, evaluation and analysis methods

Milk samples for identification of udder pathogens

In study I, the herd veterinarians registered symptoms (general condition, appetite, milk appearance, teat consistency, and teat end condition) and treatment given at every case of VTCM on the participating farms. The time from detection of CM by the farmer until the visit of the veterinarian was also registered. The veterinarians also took milk samples from mastitic udder quarters for bacteriological examination. The bacteriological diagnosis was confirmed at the National Veterinary Institute, Uppsala, Sweden. In study III, the farmers were asked to take milk samples from udder quarters of first-parity cows at a suspected (not treated) case of CM and send the samples to the National Veterinary Institute, Uppsala, Sweden, for bacteriological diagnosis.

Hygienic quality of feed

In studies I and III the hygienic quality of feed was tested. In study I, grass silage samples taken at two occasions were analysed for pH, ammonium nitrogen and sugar, as quality indicators, and for dry-matter (DM) content. Storage facilities for dry and wet feedstuffs were also evaluated. In study III grass silage and grain samples were collected once. The silage samples were analyzed for pH, DM, and mould, bacteria and yeast contents, while the grain samples were analyzed for water activity, and mould, bacteria and yeast contents.

Housing

In study I, housing and housing conditions (stall type, bedding material, cleanliness of stalls, draft, moisture *etc*.) for calves, young heifers, pregnant heifers, lactating and dry cows were observed and evaluated by the principal investigator. In study II the project veterinarian recorded the housing system for the heifers from calving to first lactation. Housing conditions were not observed or evaluated in studies III and IV, but the farmers were asked about housing systems (*e.g.* type of stalls and aisles, bedding, cleaning procedures) for first-parity cows at one month before calving, at calving, and at one month after calving.

Cow cleanliness and body condition

Cleanliness of lower fore and hind-legs, flank, belly, udder, escutcheon, tail head and tail of calves, young heifers, pregnant heifers, dry cows, cows at 3 months after calving ("high yielding" cows) and cows at 6 months after calving ("low yielding") cows were scored in study I. Body condition scores were evaluated for cows at 3 months after calving, cows at 6 months after calving, dry cows, and heifers within 3 months before calving in study I.

Milking machine function and milking routines

The milking machine function and milking routines were only tested and observed in study I. The function was tested according to the Nordic guidelines, and milking time, vacuum levels and vacuum fluctuations were measured during milking. Milking routines were recorded and a time-study of the routines applied was conducted. In studies II and III no observations or evaluations were made in the area of milking, but the farmers were asked questions *e.g.* about usage of restraining measures, post-milking teat disinfection, and adaptation of first-parity cows to milking.

Blood parameters

In study IV blood samples were taken from first-parity cows at 1 to 4 occasions per animal between -14 to 60 days after calving. All samples were analyzed for serum concentrations of NEFA, BHBA, glucose, insulin, urea nitrogen, total cholesterol and haptoglobin. For financial reasons, serum concentrations of conglutinin and CL-43 were only analyzed in the first two samples taken after calving, and serum concentrations of α-tocopherol and Se were only analyzed in one sample per animal taken -5 to 5 days after calving.

Statistical methods

Univariable and multivariable logistic regression models were used to analyse associations between herd level risk factors and being a LO- or HI-herd in paper I. In paper II, univariable and multivariable hierarchical logistic regression models were used for the statistical analyses of risk factors associated with VTCM in the period -7 to 30 days after calving and with SCC $\geq$ 200,000 cells/mL at first test-milking. In paper III, hierarchical univariable and multivariable Poisson and linear regression models were used to investigate risk factors at herd and individual level associated with udder health of first-parity cows, measured as number of first-parity cows VTCM in the period -10 to 60 days after calving; number of first-parity cows with SCC $\geq$ 200,000 cells/mL at first test-milking; and SCC at first test-milking. In all above mentioned hierarchical models herd was included as random factor. In paper IV, associations between blood parameters measured in the period around calving and in early lactation, and breed and age at first calving were analysed using multivariable hierarchical linear regression models with herd as random variable and taking into account the repeated measured blood samplings of respective cow. Associations between blood parameters and SCC were analysed using ordinary multivariable linear regression analysis with robust standard errors and adjusting for clusters within herd. In all final models a variable with a P-value ≤ 0.05 was considered statistically significant and was retained in the model.

Collinearity was assessed for variables entering the final models in all studies. Variables with a high collinearity ($r > 0.70$) were not entered in the model at the same time, and if collinear variables were significantly associated with the outcome, separate models, each containing one of the variables, were tested and the model with the best fit was kept. Potential confounders were considered in every model. A variable was considered as a confounder if the point estimates of the coefficients in a model changed > 20% when the potential confounder was added to the model.

The fit of the models reported in paper I was evaluated with the Hosmer-Lemeshow goodness-of-fit test, and by visual examination of diagnostic plots. The model fit for the models in paper II was assessed by visual examinations of plots of ranked standardized herd-level residuals and the Hosmer-Lemeshow goodness-of-fit statistics. The normality of the residuals was tested using the Shapiro-Wilk, Kolgomorov-Smirnov, Cramer-von Mises, and Anderson-Darling tests. Model validation for the analyses in papers III and IV was performed by visual examination of the normality of residuals, by visual examination of normal probability plots of the residuals and standardized residuals (Q-Q plot) as well as visual examination of the plot of standardized residuals against the fitted values. Influential points and outliers were identified by Cook's distance values, leverage and "difference in fit" (DFITS) values.

All statistical analyses were performed in SAS version 8.2 and 9.1 (SAS Institute Inc., Cary, NC) or Stata software (Stata Statistical Software: Release 9.2; College Station, TX, USA: StataCorp LP.).

Results

Descriptive data

Incidence of VTCM

The IRVTCM found for LO and HI-herds in paper I showed that the herds on average belonged to the lowest quarter or top fifth, respectively, concerning VTCM also during the year of participation. The median IRVTCM in LO-herds was 0.67 cases per 100 cow-months, while a median of 2.54 cases per 100 cow-months were found in HI-herds.

Of 2,126 first-parity cows participating in study II 229 (10.8%) were VTCM during their first lactation (305 days), corresponding to an IRVTCM of 1.13 cases per 100 cow-months. During the period -7 to 30 days after calving, 131 cases (51% of all cases during first lactation) were recorded in 64 herds.

In study III, 76 first-parity cows (6.6% of 1,150 first-parity cows in 70 herds contributing with VTCM data; range 0 - 5 cases/herd) from 42 herds were VTCM in the period -10 to 60 days after calving. This corresponds to an IRVTCM of 2.83 cases per 100 cow-months.

The results from papers I-III showed that most cases of VTCM occurred during the first weeks of lactation. Moreover, according to paper I, first-parity cows, both in LO and HI-herds, had a numerically higher IRVTCM than second-parity cows in the period around calving, and as high as that for third-parity and older cows in HI-herds.

Cow SCC

The geometric mean SCC for first-parity cows at first test-milking was 66,000 cells/mL (50% central range (CR; excluding 25% of the values at each end of the distribution): 29,000 to 138,000 cells/mL) in paper II, 64,300 cells/mL (50% CR: 29,000 – 118,000 cells/mL) in paper III, and 62,700 cells/mL (50% CR: 31,000 – 105,000 cells/mL) in paper IV. The first test-milking was on average at day 21 after calving in papers II and IV, and on day 20 after calving in paper III. Of the first-parity cows in papers II and III, 18.1% and 14.7%, respectively, had a SCC ≥ 200,000 cells/mL at first test-milking.

Isolates of udder pathogens

The udder pathogens most frequently isolated in milk samples from cases of VTCM in LO- and HI-herds in study I are shown in Figure 1 (additional results to those reported in paper I). Milk samples were taken from 778 udder quarter cases from 117 herds (641 quarter cases from 70 HI-herds; 137 quarter cases from 47 LO-herds) in study I. The sampled cases constituted 53% of all cases of VTCM (54% of the cases in HI-herds and 46% of the cases in LO-herds) reported to the SADRS. Milk samples were taken from udder quarters from 105 first-parity cows with suspected cases of CM from 41 herds in study III. In only 14% of the 242 cases of VTCM reported to the SADRS in study III, had the farmers taken milk samples. In total 67% of the sampled cases of suspected CM were not veterinary treated or veterinary treated but not reported to the SADRS.

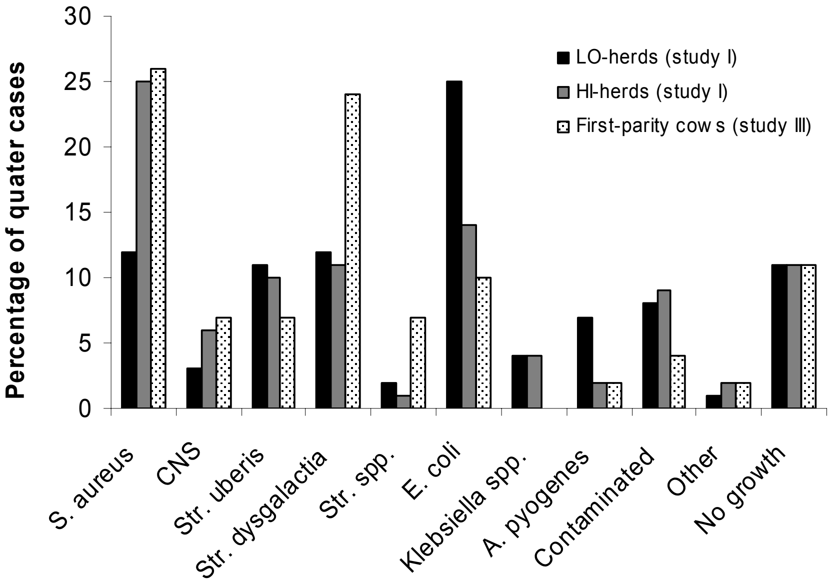

Figure 1. Micro-organisms isolated from quarter cases of veterinary treated clinical mastitis (VTCM) in dairy herds with a documented high milk yield, low prevalence of subclinical mastitis and a low (n=137 sampled quarters; 47 herds) or high (n= 641 sampled quarters; 70 herds) incidence of VTCM (paper I), and suspected quarter cases of clinical mastitis of first-parity cows (n=135 sampled quarters; 41 herds) from large, high producing Swedish dairy herds housed in free stalls (paper III).

The most frequently isolated udder pathogens in study I were *S. aureus* (22%), *E. coli* (16%), *Str. dysgalactiae* (11%), and *Str. uberis* (10%). In 11% of the cases no micro-organisms were isolated. Proportionally more cases were found positive for *S. aureus* in HI-herds ($P < 0.005$), while proportionally more cases were found positive for *E. coli* ($P < 0.005$) and *A. pyogenes* ($P < 0.05$) in LO-herds. The distribution of micro-organisms isolated at suspected quarter cases of CM of first-parity cows from the herds in study III (additional results to those reported in paper III) are also shown in Figure 1. The most common isolates were *S. aureus* (26%), *Str. dysgalactiae* (24%), *E. coli* (10%), and *Str. uberis* (7%). In 11% of the cases no micro-organisms were isolated. The distribution of udder pathogens in first-parity cows were more similar to that of cows from HI-herds than for cows of LO-herds in study I, but the proportion of cases of *Str. dysgalactiae* was higher in the study of first-parity cows.

Herd and cow characteristics

Herd size, annual milk yield and percentage of cows with UDS 0 to 2 during the year of participation for all the studies are presented in Table 2.

Table 2. *Herd characteristics for herds participating in four studies of factors associated with udder health of dairy cows in Sweden (1997-2006). Median values (1st and 3rd quartiles) are presented*

	Year of participation	Number of herds	Herd size, cow/year	Milk-yield, kg/cow-year	Proportion of cows with UDS[a] 0 to 2
Paper I	1997-1999				
HI-herds		79	32 (27; 47)	9,292 (8,843; 9,777)	71.6 (67.7; 76.7)
LO-herds		79	37 (30; 44)	8,789 (8,455; 9,263)	69.5 (64.5; 76.1)
Paper II	1998-2001				
Herds from Skaraborg county		107	51 (39; 66)	9,127 (8,597; 9,741)	61.7 (53.1; 66.9)
Paper III	2005-2006				
Large herds in free stalls		72	149 (107; 173)	10,090 (9,490; 10,600)	60.1 (52.8; 64.9)
Paper IV	2005-2006				
Subset of herds from paper III		20	205 (135; 234)	10,290 (9,430; 11,100)	57.4 (52.8; 62.6)

[a]Animals with UDS 0 to 2 have a probability of 0–29% that one or more udder quarters are infected. The score corresponds approximately to having a SCC of < 130,000 cells/mL on three consecutive test-milkings.

The mean annual milk production ($P < 0.001$), percentage of cows with an UDS of 0 to 2 ($P < 0.10$), and IRVTCM ($P < 0.0001$) during the year of participation were higher in HI-herds compared to LO-herds (paper I).

In total, 35% of the herds in study I had a herd mainly consisting of SR-cows, while 30% mainly had SH-cows, and 35% had cows of both breeds. In study II, 49% of the first-parity cows were of the SR-breed, 49% were of the SH-breed, and 2% were crossbred (SH/SR). Of the herds in study III 44% were classified as SH-herds, 15% as SR-herds and 40% as SH/SR-herds. On the individual level 60%, 36% and 4% first-parity cows were SH, SR and crossbred (SH/SR), respectively (paper III). The majority of the first-parity cows in study IV was SH (65%), while 30% were SR, and 5% were crossbred (SH/SR).

The majority of farmers (90%) participating in the studies reported in papers I-II housed their cows in tie stalls. The housing system did not differ between LO- and HI-herds in paper I ($P > 0.25$). All farmers housed their lactating cows in free stalls in studies III-IV, which was one of the inclusion criteria. Most first-parity cows (62%) in study II were housed in tie stalls two months before parturition, while the rest of the first-parity cows were housed in free stalls, group boxes or in a loose housing system. In contrast, 74% of the herds in study III housed their first-parity cows in free stalls, while the remaining herds used tie stalls one month before calving. In most herds (68%) first-parity cows calved alone in a calving box, while group-calving boxes were used in 17% of the herds (paper III). The first-parity cows were moved to the calving area ≤ 1 day before parturition in 61% of the herds, and from the calving area ≤ 1 day after parturition in 54% of the herds.

In studies I-II, the most common (85-90% of the herds) feeding system were to feed roughage and concentrates separately, and not as a total mixed ration (TMR). At one month before calving the first-parity cows were fed a partially mixed ration (PMR) in 19% of the herds, TMR in 29% of the herds, or grass silage and concentrates separately in 51% of the herds (paper III). At calving, the first-parity cows were fed PMR in 41% of the herds, TMR in 28% of the herds, and roughage and concentrates separately in 31% of the herd. In 54% of the herds participating in study III, the lactating cows were fed PMR, while 25% of the herds fed a TMR, and 21% fed grass silage and concentrates separately. The percentage roughage used in the total ration to first-parity cows in study III was 84% at one month before calving, 62% at calving, and 40% at one month after calving.

Grass silage was used in all farms in all studies. Other roughages used in combination with grass silage were *e.g.* corn silage, hard pressed (HP) sugar-beet pulp, hay, straw and whole grain silage. Corn silage was used in 22% of the herds in study III, while none of the herds in study I used corn silage. Hard pressed sugar-beet pulp (27% DM) was used in 9% and 44% of the herds in studies I and III, respectively. None of the herds in study II had any registrations of usage of corn silage or HP sugar-beet pulp (27% DM).

Roughage was most commonly used in combination with grain (barley, oats, wheat and/or ray-wheat) and an industrially processed concentrate. Soy was used in 2%, 12%, and 21% of the herds in studies I-III, respectively. In approximately

70% of the herds in studies II-III, the adaptation of the first-parity cows to the lactating cow diet started ≥ 3 weeks before calving, and in the rest of the herds it started < 3 weeks before calving. In study I, the mean amount of concentrates given to multiparous cows at calving and at one month after calving was 4.8 kg and 16.6 kg, respectively. The mean amount of concentrates given to first-parity cows at calving in studies I-III was 4.1 kg, 4.0 kg, and 5.7 kg, respectively. In studies I and III first-parity cows were given 13.6 kg and 12.8 kg of concentrates, respectively, at one month after calving. Additional minerals were given in 92-97% of the herds, while vitamins, mostly vitamins A, D, and E in combination or vitamin E solely, were given in 15-17% of the herds.

In studies I-II approximately 80% of the farmers milked their cows in tie stalls, while the remaining farmers milked their cows in a parlor. In study III the majority of farmers (approximately 80%) milked their cows in a parlor, while the rest used a rotary (8%) or a voluntary milking system (10%). In 43% of the herds participating in study III the first-parity cows were allowed to go to the parlor before calving to get used to the milking routines, while no adaptation to milking was applied in 40% of the herds. Post-milking teat disinfection was used in 98% and 87% of the herds in studies I and III, respectively. Usage of post-milking teat disinfection was not registered in study II.

Hygienic quality of feed

The mean dry matter (DM) content, pH, and sugar content of the grass silage samples taken in study I was 33% (50% CR: 27% – 35%), 3.3 (50% CR: 1.0 – 4.1), and 0.001% (50% CR: 0.0% – 0.001%), respectively. The results of the ammonium-nitrogen analysis were not considered reliable and are not presented.

The mean DM content of the grass silage samples taken in study III was 31% (50% CR: 26% – 34 %), while the mean pH was 4.2 (50% CR: 3.9 – 4.3). Growth of yeast and mould was observed in 69% and 72% of the grass silage samples, respectively. Of all the grass silage samples 44% had growth of yeast over the accepted limit set by the laboratory, while 23% had growth of mould over the accepted limit. In 40% of all the grass silage samples *Penicillium (P.) roqueforti* was found.

Grain was stored at 74% of the farms in study III, and the mean DM content of the grain samples was 72% (50% CR: 66% – 77%). Growth of yeast and mould were observed in 94% and 92% of the grain samples, respectively. The accepted limit of yeast findings was exceeded in 26% of the grain samples, while the accepted limit for growth of mould was exceeded in 41% of the grain samples. *Aspergillus* spp. and *Fusarium* spp. was found in 18% and 82%, respectively, of all grain samples.

Multivariable models and major findings

In study I 363 variables were screened in the initial univariable analysis, 75 of those had a P-value of ≤ 0.25, 35 were significantly ($P < 0.10$) associated with the IRVTCM, and 13 remained in the final model ($P < 0.05$).

In the initial univariable analysis in study II of factors associated with udder health of first-parity cows measured as VTCM in the period -7 to 30 days after calving, and as having SCC ≥ 200,000 cells/mL at first test-milking, 98 herd-level and 10 cow-level variables were screened. Of those variables 12 and 7, respectively, were considered in the final multivariable analysis, while 2 and 4, respectively, remained in the final models ($P < 0.05$) of factors associated with the specified udder health outcomes.

A total of 152 herd-level and 11 cow-level variables were screened in the initial univariable analyses for associations with the three udder health outcomes in study III. In total 32, 23, and 27 variables had a P-value of ≤ 0.20 for the analyses of factors associated with VTCM, SCC ≥ 200,000 cells/mL at first test-milking, and SCC (transformed to the natural logarithm of SCC (lnSCC)) at first test-milking, respectively. Of those variables, a total of 9, 7, and 11, respectively, remained in the sub-models ($P < 0.10$), while 5, 3, and 6 variables remained in the final models ($P < 0.05$) of the three outcomes, respectively.

In the modeling of associations between metabolites, immune variables, and SCC of first-parity cows at first test-milking in study IV 13 variables entered the final model, of which 8 remained ($P < 0.05$).

Herd and cow factors

The following herd and cow factors were found significantly associated with udder health in the final statistical models:

- To have a herd with ≥ 80% of the cows of SR-breed was associated with being a herd with a low IRVTCM (paper I)
- To have a high proportion of first-parity cows in the herd was associated with being a herd with a high IRVTCM (paper I).
- To be a first-parity cow of the SR-breed was associated with having lower SCC at first test-milking (paper III).
- To be a first-parity cow with a high milk-yield at first test-milking was associated with lower SCC at first test-milking (paper III).
- To be a first-parity cow with a milk urea < 4 mmol/L compared to 4 mmol/L was associated with higher SCC at first test-milking (paper III).
- To be a first-parity cow with a milk urea ≥ 5 mmol/L compared to 4 mmol/L was associated with lower SCC at first test-milking (paper III).

Health

The following health related factors were found significantly associated with udder health in the final statistical models:

- To have veterinary-treated teat injuries in a herd was associated with being a herd with a high IRVTCM (paper I).
- To be a first-parity cow in a herd with high IRVTCM was associated with an increased risk of being VTCM in the period -7 to 30 days after calving (paper II).

- To be a first-parity cow with reproductive disorders (retained placenta, endometritis, pyometra, dystocia or twin birth) was associated with an increased risk of being VTCM in the period -7 to 30 days after calving (paper II).
- To be a first-parity cow in a herd with a high proportion of cows with UDS 6 to 9, at least once during the lactation, was associated with increased risk of having a SCC ≥ 200,000 at first test-milking (paper II).
- To be VTCM before first test-milking and then having a milk-fat percentage ≥ 4.2% at first test-milking was associated with being a first-parity cow with higher SCC at first test-milking (paper III).

Preventive health measures

The following preventive health measure related factors were found significantly associated with udder health in the final statistical models:

- To treat cows with high SCC during lactation with antimicrobials was associated with being a herd with a high IRVTCM (paper I).
- To have a high proportion of cows dry-cow treated with antimicrobials was associated with being a herd with a high IRVTCM (paper I).
- To claw-trim cows in the herd more than once per year was associated with being a herd with a high IRVTCM (paper I).
- To not contact the veterinarian for treatment of a cow with CM until the cow shows systemic signs of illness was associated with being a herd with a low IRVTCM (paper I).

Feed and feeding

The following feed and feeding factors were found significantly associated with udder health in the final statistical models:

- To have one or more remarks on the condition of grass silage storage was associated with being a herd with a high IRVTCM (paper I).
- To give only industrially processed concentrates was associated with being a herd with a low IRVTCM (paper I).
- To be a first-parity cow in a herd where heifers at the age 11 to 16 months were given high amounts of concentrates (kg/day) was associated with an increased risk of having a SCC ≥ 200,000 cell/mL at first test-milking (paper II).
- To give sugar-beet pulp from calving and onwards to first-parity cows was associated with increasing within-herd number of first-parity cows VTCM in the period -10 to 60 days after calving (paper III).
- To give grass silage from a different batch to pregnant heifers than to lactating cows was associated with higher within-herd number of first-parity cows VTCM in the period -10 to 60 days after calving (paper III).
- To give corn silage from calving and onwards to first-parity cows was associated with higher within-herd number of first-parity cows with SCC ≥ 200,000 cells/mL at first test-milking (paper III).

- To give sugar-beet pulp at one month before calving and onwards to first-parity cows was associated with higher SCC at first test-milking (paper III).

Housing and pasture

The following housing and pasture related factors were found significantly associated with udder health in the final statistical models:

- To have high-yielding cows with dirty lower hind-legs was associated with being a herd with a high IRVTCM (paper I).
- To move or house cows as a routine at drying off was associated with being a herd with a low IRVTCM (paper I).
- To be moved from pasture to confined housing at the day of calving as first-parity cow was associated with an increased risk of having a SCC ≥ 200,000 at first test-milking (paper II).
- To house pregnant heifers in tie stalls one month before calving was associated with lower within-herd number of first-parity cows VTCM in the period -10 to 60 days after calving (paper III).
- To use sawdust or shavings as bedding material compared to straw in the calving area was associated with higher within-herd number of first-parity cows VTCM in the period -10 to 60 days after calving (paper III).
- To be moved from the calving area ≥ 2 days after calving compared to < 2 days after calving as first-parity cow was associated with having high SCC at first test-milking (paper III).
- To have mattresses as flooring material in cubicles in the lactating cow housing compared to nothing or rubber mats was associated with being a first-parity cow with lower SCC at first test-milking (paper III).

Milking

The following milking factors were found significantly associated with udder health in the final statistical models:

- To use certain restraining measures of first-parity cows during milking was associated with increased risk of first-parity cows having a SCC ≥ 200,000 at first test-milking (paper II).
- To milk first-parity cows in the calving area instead of in the parlor during the colostrum period was associated with higher within-herd number of first-parity cows VTCM in the period -10 to 60 days after calving, and higher within-herd number of first-parity cows with SCC ≥ 200,000 cells/mL at first test-milking (paper III).
- To rinse, clean or disinfect milking units before a first-parity cow is milked was associated with higher within-herd number of first-parity cows with SCC ≥ 200,000 cells/mL at first test-milking (paper III).

Blood parameters associated with SCC

The following blood parameters were found significantly associated with Box-Cox transformed SCC (bcSCC) of first-parity cows at first test-milking in the final statistical models (paper IV):

- To be a first-parity cow with higher levels of BHBA, cholesterol and glucose before calving was associated with lower bcSCC.

- To be a first-parity cow with higher levels of NEFA before calving was associated with higher bcSCC.
- To be a first-parity cow with higher delta NEFA (NEFA after calving minus NEFA before calving) was associated with higher bcSCC.
- To be a first-parity cow with higher levels of α-tocopherol in the period -5 to 5 days after calving was associated with lower bcSCC.
- To be a first-parity cow with higher levels of CL-43 and haptoglobin postpartum (1 to 21 days after calving) was associated with higher bcSCC.

Associations between days from calving, breed, age at calving and blood parameters

The results of the statistical analyses of associations between days from calving (period), breed, and age at calving, and BHBA, cholesterol, CL-43, conglutinin, glucose, haptoglobin, insulin, NEFA, and urea nitrogen (paper IV) showed that period was significantly associated with all parameters, but as an interaction term with breed for cholesterol, NEFA, and CL-43. Breed as main effect was significantly associated with insulin, urea nitrogen, and conglutinin. In general, first-parity cows of the SR-breed had higher concentrations of cholesterol, conglutinin, insulin, and urea nitrogen, and lower concentrations of NEFA and CL-43 than cows of the SH-breed.

Age at calving as main effect was significantly associated with BHBA, glucose, insulin, NEFA, urea nitrogen, and conglutinin. In general, heifers calving at an older age (> 27 months) had higher BHBA and NEFA, and lower glucose, insulin and urea nitrogen values than heifers calving at a younger age (< 27 months). Heifers calving at an even younger age (< 25 month) had higher conglutinin and urea nitrogen, and lower NEFA values compared to heifers calving at an older age (> 25 months). Heifers aged < 25, and 25-27 months at calving had similar BHBA, glucose, and insulin values, while heifers aged 25-27, and > 27 months at calving had similar conglutinin values.

Discussion

Detailed discussions of the findings in the multivariable analyses are found in the respective paper. Below, a summarizing discussion of some of the major findings follows.

Cows of the SR-breed have better udder health

In both papers I and III the results showed that herds and cows of the SR-breed had better udder health than herds and cows of the SH-breed. This relationship has been reported repeatedly during the last decades (Bendixen *et al.*, 1988; Emanuelson, Oltenacu & Gröhn, 1993; Swedish Dairy Association, 2003; Swedish Dairy Association, 2006). Other studies have also shown a significant association between breed and IRVTCM at herd level (Schukken *et al.*, 1990; Elbers *et al.*, 1998), and between breed and SCC on individual level (De Vliegher *et al.*, 2004a; Compton *et al.*, 2007b), but no studies have identified the biological background to these differences. Possible explanations could be related to morphological differences, such as udder and teat shape, but also to differences in immune function. Evidence of such breed differences between SR- and SH-cows were found in study IV. The metabolism and immune function in the period around calving appeared to be more favorable in SR-cows than SH-cows. This was indicated by differences in the serum pattern of substances known to be related to metabolism and immune function (paper IV). It appears as first-parity cows of the SR-breed cope better than SH-cows with the changes around calving and in early lactation, which in turn is favorable for udder health. The relationship between nutrition, immune functions and udder health is complex and more research is needed to clarify these associations.

First-parity cows are at increased risk of udder disorders at calving and in early lactation

At individual cow-level the risk of CM increases with parity/age (Dohoo & Martin, 1984a; Syväjärvi, Saloniemi & Gröhn, 1986; Bendixen *et al.*, 1988; Emanuelson, Oltenacu & Gröhn, 1993). However, in paper I we found an association between being a herd with a high IRVTCM and increasing proportion of first-parity cows in the herd. The first-parity cows in study I contributed to a large proportion of the VTCM, especially during the first week after calving. During this period first-parity cows in HI-herds had a numerically higher IRVTCM than second-parity cows, and as high as older cows (third parity cows or older). A higher incidence of CM in first-parity cows compared to in older cows in the beginning of lactation has also been reported by others (Barkema *et al.*, 1998b; Valde *et al.*, 2004). To suffer from clinical mastitis early in lactation will have negative effects on udder health and production throughout the lactation. Clinical mastitis in early lactation increases the risk of mastitis later in lactation (Edinger *et al.*, 1999; van Dorp *et al.*, 1999), and an elevated SCC in early lactation often results in elevated SCC throughout the whole lactation (De Vliegher *et al.*, 2004a).

Monitoring first-parity cows more, especially at calving and in early lactation, reducing interactions with dry-cows with unknown udder-health status both before and at calving, providing them with a clean calving area and feed of good nutritional and hygienic quality will, according to the results presented in this thesis, reduce their risk of udder disorders.

Reproductive disorders in early lactation increase the risk of CM

Results from study II, and from a separately performed analysis of factors associated with being a first-parity cow in a LO- or HI-herd from the material in study I (Arvidson *et al.*, 2005), showed that first-parity cows veterinary treated for reproductive disorders at calving, *e.g.* retained placenta, pyometra, endometritis, were at greater risk of also being VTCM. Other studies have also shown associations between retained placenta and CM (Bendixen *et al.*, 1988; Gröhn *et al.*, 1990; Oltenacu & Ekesbo, 1994). If reproductive diseases in the period around parturition are prevented, the risk of CM would probably be reduced considerably. Supplementation with vitamin E and Se during the dry period is associated with enhanced immune functions (Hogan *et al.*, 1992; Cebra *et al.*, 2003), and some have found beneficial effects of vitamin E and Se supplementation both on retained placenta (reviewed by Wilde, (2006)) and on udder health (Smith *et al.*, 1984; Weiss *et al.*, 1990). In study III, we showed an association between higher serum α-tocopherol levels in the period around calving and lower SCC of first-parity cows at first test-milking. However, others have not been able to find an association between increased supplementation of vitamin E and udder health (Erskine *et al.*, 1997; Persson Waller *et al.*, 2007). Kommisrud, Østerås & Vatn (2005) found that a moderate supplementation of Se was beneficial both for udder health and retained placenta while both low and high supplementation levels of Se were negative for these disorders. There seems to be a complex relationship between udder health, retained placenta, vitamin E and Se, which needs to be investigated further. However, in herds with high incidences of retained placenta, supplementation of vitamin E and Se in late pregnancy and in early lactation will probably be beneficial, reducing incidences of retained placenta as well as CM.

Improved udder health with feed of good hygienic quality

The results indicate that the hygienic quality of feed is one of the few common denominators found in the studies presented in this thesis. Both silage and grains can be contaminated with undesirable micro-organisms, which alter the nutrient value and hygienic quality of the feed. The effect of these micro-organisms and/or their influence on the feed and its association with health of animals has been reviewed by Wilkinson (1999). In paper I, the storage conditions for silage in HI-herds had more remarks than the storage conditions in LO-herds. Bad storage condition increases the risk of deterioration of feed quality, which in turn may have direct or indirect negative effects on the immune system, and hence, increase the risk of mastitis. The finding in paper III, that giving first-parity cows silage from a different batch before and after calving was associated with increasing within herd number of first-parity cows VTCM, can also be related to hygienic quality of feed. It is more likely that feed from a batch of poorer quality is given to other groups of animals than to lactating cows. However, we do not know if the silage given from a different batch before calving necessarily had inferior quality compared to the batch given after calving.

Another finding possibly related to the hygienic quality of feed is the finding in paper I that it was more common to only give industrially-processed concentrates in LO-herds than in HI-herds. Industrially-processed concentrates are presumed to be properly dried and stored, and are almost always heat treated. These measures are important in order to achieve concentrates with good hygienic quality. Home-dried concentrates (grains) may differ more in quality due to lack of drying capacity and suitable storage facilities. Consequently, clean and dry storage facilities, and thorough drying of grains can be factors improving udder health on a farm.

The findings in paper III, that usage of corn silage or HP sugar-beet pulp were associated with poorer udder health have been found in other studies, but none have been able to explain the nature of these associations (Schukken *et al.*, 1990; Barkema *et al.*, 1999; Bareille *et al.*, 2000). However, Auerbach, Oldernburg, & Weissbach (1998) detected *P. roqueforti* in more than 80% of sampled silages (both corn and grass silage) taken from different dairy farms in Germany, and Nout *et al.* (1993) detected *P. roqueforti* in 40% of the HP sugar-beet pulp samples received from Dutch farms. In comparison, we found *P. roqueforti* in 40% of the sampled grass silages in study III. The corn silage and HP sugar-beet pulp was not sampled in study III, but it is most likely that we would have found *P. roqueforti* also in those samples since *P. roqueforti* is the dominant spoilage mould of corn silage and HP sugar-beet pulp (Nout *et al.*, 1993). Higher concentrations of the mycotoxin roquefortin C were found in mould-affected corn silages than in mould-affected grass silages (Auerbach, Oldernburg & Weissbach, 1998), and the authors speculate that the high content of highly soluble carbohydrates in corn silage could increase the production of roquefortin C. Sugar-beet pulp does also contain large amounts of highly soluble carbohydrates, indicating a similar risk of finding high concentrations of roquefortin C if *P. roqueforti* infected. Even low levels of mycotoxins are believed to cause immune suppression and increase the risk of infectious diseases (Osweiler, 2007), emphasizing the importance of using feed of high hygienic quality.

The amounts of concentrates given influence udder health

In studies I-III the amounts of concentrates given at different periods were found associated with udder health and were offered to the final models. Higher amounts of concentrates given at calving and at peak milk production were associated with being a HI-herd (paper I), and higher amounts of concentrates given to heifers at 11-16 months of age increased the risk of having a SCC ≥ 200,000 cells/ml at first test-milking (paper II), while higher amounts of concentrates given one month after calving was associated with a reduced number of first-parity cows VTCM (paper III). Other studies have also found associations between concentrate feeding and CM, but as in our case, these variables are most often not retained in the final multivariable models (Fraser & Leaver, 1988; Schukken *et al.*, 1991; Waage, Sviland & Odegaard, 1998; Barkema *et al.*, 1999; Barnouin *et al.*, 2005). The relationship between amounts of concentrates given and mastitis seems complex as higher amounts have been associated both with lower and higher incidences of CM. Moreover, the association seems to differ depending on when (age or lactation stage) the feed is given. Thus, this area needs more research, and other factors like type and chemical composition of the feed, breed, and genetic merit for milk production must be taken into consideration when studying associations between amounts of concentrates and mastitis.

The importance of reducing stress in the period around calving

One of the hypotheses in study III was that an increased number of transfers and changes of housing and bedding material in the period around calving would be stressful and negatively influence the udder health of first-parity cows. However, we could not confirm this hypothesis, perhaps due to the measurement methods used. Number of transfers may be a too rough measurement when used at herd level, since there is an individual variation in sensitivity to stressors (Mallard *et al.*, 1998). A more sensitive measurement at herd level might be the number of animals with altered grooming behaviour after moving to new housing/group/bedding, which could be investigated by video recording. The grooming behaviour of cows has been shown to be altered when cows are moved and introduced into a new group of animals (von Keyserlingk *et al.*, unpublished). Indications of associations between potential stressors and udder health was found in paper II where housing the day of calving of first-parity cows on pasture, as well as restraining methods used at milking, were associated with being a first-parity cow with SCC ≥ 200,000 cells/mL at first test-milking. As reviewed by Persson Waller (2000) there are a number of potential stressors influencing udder health, but methods to record these stressors need further development.

Important udder pathogens and practical considerations of milk sampling

Milk samples for isolation of udder pathogens at cases of VTCM, or at a suspected case of CM were taken in studies I and III. However, when comparing cow-id and date of occurrence of a case of VTCM from the records from the SADRS with the referral sent in with the milk samples dissimilarities were found. The most common reason for the dissimilarities was the fact that milk samples were not sent to the laboratory (common in study I), but also that some milk samples came from cases not reported to the SADRS (common in study III). However, the distribution of isolated bacteria in studies I and III, mainly isolates of *S. aureus*, *Str. dysgalactiae*, *E. coli*, and *Str. uberis*, are in accordance with findings from a recent nationwide Swedish investigation of prevalence of bacteria at acute cases of VTCM (Bengtsson *et al.*, 2005; Persson Waller, 2007). The distribution is also similar to findings in a Dutch study (Barkema *et al.*, 1999), though somewhat different from findings by Schukken *et al.* (1990) and Miltenburg et al (1996) where *E. coli* was the most common finding at a case of CM. Waage *et al.* (2001) found more isolates of CNS, and less of *E. coli*, at cases of clinical mastitis compared to our findings. Miltenburg *et al.* (1996) found that first parity-cows had more isolates of *Str. dysgalactiae* than older cows, which is comparable to our findings. *Str. dysgalactiae* have been isolated *e.g.* from the tonsils of cows, and opportunities of intersucking, for example when housing heifers in a group before calving, might increase the risk of *Str. dysgalactiae* infections. In paper III we found a reduced within herd number of first-parity cows VTCM in herds where first-parity cows were in tie stalls before calving, which reduces the risk of intersucking. However, housing type before calving (tie stalls vs. free stalls) did not influence the prevalence of *Str. dysgalactiae* infections found in study III.

S. aureus was the most common isolate at cases of VTCM in HI-herds in study I and at suspected cases of CM of first-parity cows in study III. *S. aureus* is classified as a contagious micro-organism, which often spreads between cows at milking, and it is often difficult to eliminate by antimicrobial treatment. In study I, *S. aureus* was proportionally more often isolated at a case of CM in HI-herds than in LO-herds, while proportionally more *E. coli* was isolated at a case of CM in LO-herds compared with HI-herds. This finding indicates difficulties in stopping the spread of *S. aureus* in HI-herds. Results from a Swedish study by Persson Waller *et al.* (2007) showed that *S. aureus* was common also in VTCM cases from first-parity cows occurring the first days after calving. This indicates that many first-parity cows are infected already before or at calving. A number of risk factors for *S. aureus* infections in heifers have been reported such as colonization on teat skin or in the inguinal area, transmission with flies and keeping heifers with older cows (Sears & McCarthy, 2003) Research investigating presence of *S. aureus* at various sites (both on animals and in the environment) in herds with *S. aureus* problems is conducted in Sweden at present (Capurro *et al.*, unpublished). The results may hopefully point out important risk areas.

Even though the distribution of micro-organisms was in accordance with other studies, the number of samples was too low to include in the statistical analyses in the studies (I and III). It is difficult to solve the problem of collecting milk samples from cases of CM, which in our case depended on the interest of the veterinarians and farmers to send in milk samples. Engaging the veterinarians more in the research project, *e.g.* through planning meetings, might increase the number of milk samples sent in at a case of CM in these kinds of studies. However, farmers in Sweden often use several veterinarians, sometimes from different organisations, making it hard to engage all veterinarians visiting the farm. Moreover, farmers participating in a study are often very interested, but their work-load may force them to not prioritise collection of milk samples. In contrast, some farmers might send in too many milk samples, also sending in samples from cows with high SCC, but with no visible clinical signs. The best solution might be to try to involve both the farmers and the herd veterinarians more, encouraging them to send in samples, and to document the clinical signs better.

Methodological considerations

The associations identified in the multivariable analyses did not fully agree between the different studies probably due to differences in study design, outcome measures, questions asked, measurements used, and limitations of the statistical procedures. Moreover, some of our hypotheses about an influence of housing, milking technique and equipment, and stress related management (such as moving cows several times in the period around calving) on mastitis could not be proven or discharged. Those hypotheses could still be valid, but may need other study designs, other ways of measure the factors of interest, or identification of other factors that are more representative of the area of interest, to be fully examined.

Study populations and study designs

Choice of sample size is an important step when planning a study and it involves both statistical and non-statistical considerations. The statistical considerations include various elements such as required precision of the estimate, expected variance in the measurements used, desired level of confidence, and the power to detect real effects in the study (Dohoo *et al.*, 2003). However, limitations are often set by non-statistical elements; time limitations, financial limitations, availability of study objects *etc*. Financial constraints limit the number of observational units (herds, cows, blood samples *etc.*), time for observations, and analytical methods used. In the present studies, the number of herds/cows participating, and the number of feed and blood samples analysed was decided based on economical and practical reasons. Sample sizes used in similar studies were also considered and influenced the decisions. The power of the studies was not calculated, but was most likely not so high (< 0.80), which reduces the chances of detecting significant findings. This can explain the fact that some factors expected to be associated with udder health were not found significant in the present studies. Nevertheless, several factors were still found significantly associated with udder health. However, when many factors are studied in large datasets, the possibility of finding associations due to chance alone increases (Dohoo *et al.*, 1997). To be certain that the associations found in the studies presented in this thesis are true, the results should be confirmed by previously published independent studies, or by further studies.

The study populations used in the studies in this thesis are not representative of all Swedish dairy herds, but of herds, both in Sweden and in other countries, with similar characteristics. At the time of selection herds in papers I, III, and IV were selected to be representative of future dairy herds to make the results of these studies useful in a longer perspective. The study population used in paper II was selected on the basis of the housing system for calves and replacement heifers, and is representative of herds with those housing systems.

When selecting study objects for a study, and asking them to participate, there is a risk of selection bias due to the fact that the willingness to participate also can be reflected in the factors studied (Rothman & Greenland, 1998). In study I it was easier to get farmers of HI-herds to participate than farmers of LO-herds. All 172 herds fitting the selection criteria of being a LO-herd had to be contacted in order to get 80 participants, while only 60% of the 202 herds fitting the selection criteria of being a HI-herd had to be contacted. However, the risk of this type of selection bias was reduced as we enrolled as many LO- as HI-herds. To reduce the risk of diagnostic bias, by misclassifying LO-herds as HI-herds and vice versa, we asked the farmers at first contact if the IRVTCM in the SADRS was correct before asking them to participate. The final model presented in paper I had a high sensitivity (87%) and specificity (86%), with an area under ROC curve of 0.93, in classifying LO- and HI- herds correctly, which gives credibility to the final model of that study.

In study II the willingness to participate, the ability to keep records, and the expressed determination to stay in milk production for at least 5 years might have resulted in the enrolment of more ambitious farmers with a specific interest in calf health, causing some selection bias. However, the study depended on the farmers ability to fulfil the aims of the study, and hence, this bias could not be avoided. As for study II the farmers accepting to participate in studies III-IV were probably more ambitious and interested in the subject of research than those declining the offer to participate, but also in this study the willingness to participate was crucial for the study. However, the 105 herds fulfilling the selection criteria in study III were given a random number (hopefully eliminating some of the risk of selection bias), and contacted according to that number. Of the 98 contacted farmers 80 agreed to participate, and the sample was thus almost a census. In studies II and III, a diagnostic bias was probably completely avoided when using the outcome measure ≥ 200,000 cells/mL at first-test milking since this is based on the true measure of SCC at that test-milking. For the outcome number of VTCM, however, there was a larger risk of misclassification, as it depends on the willingness of the farmer to contact a veterinarian for treatment, and on the veterinarians correctly reporting the case to the SADRS, which will be discussed more below.

The choice of the study designs of the present studies (case-control and cohort studies) was based on facts that these designs have a moderate to high relevance to the "real-world" situation, and have a moderate to high strength of proof of causal association (Dooho *et al.*, 2003). Though difficult to perform and the risk of confounding, field studies have an advantage versus experimental studies as cows are naturally infected and the findings can directly be applied on farms.

Using VTCM as measure of udder health

The reason for using number of VTCM and IRVTCM as outcome measures and not using number of CM or IRCM is that records of VTCM are easily available through the SADRS. Both VTCM and CM depend on the actual observation of a case of mastitis. Thus, both the nature of the case (*e.g.* severity) and the awareness of the farmer/worker can influence the number of cases being observed and reported. However, for the measure VTCM the willingness/need to contact a veterinarian for treatment, and the reliability of the veterinarian to record the treatment also influences the number of cases reported. According to a recent study of the validity of the SADRS, comparing farmer reported cases of CM with the records in the SADRS, the farmers contacted a veterinarian for treatment of CM in 78% of the cases observed at the farm, and of those 84% were reported to the SADRS (Mörk *et al.*, unpublished). However, there was a large variation in the number of cases observed at the farm that were not veterinary treated and reported to the SADRS (Mörk, 2007, personal communication). Neither veterinary reported, nor farmer reported, cases of CM is a perfect measure of the number of CM in a herd, both missing some cases and including some non-clinical cases, but limitations in technique, time, personnel, and financing make it difficult to get a more precise measure. Nevertheless, using either CM or VTCM as a measure of udder health will give information on udder health status of a cow and in a herd.

Some of the cases registered as VTCM could have been veterinary treated subclinical cases of mastitis. In Sweden, however, the policy for use of antibiotics recommends veterinarians not to treat subclinical cases with antibiotics during lactation. In the study reported in paper I the HI-farmers more often stated that they treated cows due to high SCC during lactation, hence, some of their registered cases of CM were probably subclinical. However, according to the registration forms used by the veterinarians treating cases of CM in that study, the milk appearance was slightly or severely altered in 90% of the cases, which indicates that most cases were clinical. The incidence of VTCM in LO-herds probably reflects a truer incidence of CM since the LO-farmers more often stated that they waited to contact a veterinarian for treatments until the cow showed more clinical signs, and fewer LO-farmers stated that they treated cows with high SCC during lactation. However, since the LO-farmers more often used massage and frequent milking as an alternative to veterinary treatments at a CM (results not shown) they could have had a slightly higher incidence of CM than the number of VTCM recorded. The risks of misclassification of cases (type I and II error) of VTCM mentioned above also apply to the outcomes in papers II and III. Despite these considerations, the outcome VTCM is a useful tool in studies aiming at finding factors of importance for the improvement of udder health of dairy cows, and at reducing excessive use of antibiotics. The outcome VTCM is, however, somewhat more associated with the characteristics of the farmer (attitudes towards treatments *etc.*) than the outcome CM, which is beneficial when *e.g.* trying to reduce excessive use of antibiotics.

The IRVTCM in the studies presented in this thesis were in the range of 0.67-2.83 cases per 100 cow-months, and are in accordance with other similar studies. In low SCC herds (< 150,000 cells/mL) Schukken *et al.* (1990), Barnouin *et al.* (2005), and O´Reilly *et al.* (2006) reported that the IRCM was 1.56, 1.65, and 3.03 cases/100 cow-months, respectively. In herds not selected for low SCC, IRCM have been reported to be from 1.04 to 4.19 quarter cases/100 cow-months (Elbers *et al.*, 1998; Barkema *et al.*, 1999; Waage *et al.*, 2001). In study III, the IRVTCM was rather high in comparison to studies I and II, which probably is explained by the observation period being short (71 days) and occurring in the period when the majority of cases occurs.

Using different SCC measurements as indication of udder health

As different measures of CM represent different, though similar, measures of udder health, so does different measures of SCC. This was clearly visible in paper III, where completely different factors were associated with SCC measured as continuous or dichotomized in the final models. To get an objective measure of cases of suspected subclinical mastitis we chose to dichotomize SCC at first test-milking at the cut-off of ≥ 200,000 cells/mL. A SCC of ≥ 200,000 cells/mL is a strong indication of an infection of the udder, especially of first-parity cows. Dohoo and Leslie (1991), showed that the sensitivity and specificity of distinguishing a cow infected with major pathogens (*S. aureus*, non-agalactiae streptococci, gram-negative rods and other major pathogens) from a non-infected cow at a cut-off of ≥ 200,000 cells/mL were high (> 0.84). De Vliegher *et al.* (2004a) argue, however, that the cut-off of ≥ 200,000 cells/mL is too high for detecting all IMI of first-parity cows. Using a cut-off of ≥ 200,000 cells/mL will on the other hand most certainly find those first-parity cows with an IMI caused by major pathogens. The SCC at first test-milking in the present study (63,000 – 66,000 cells/mL) was slightly higher than the findings of De Vliegher *et al.* (2004a), where the first-parity cows had a geometric mean SCC of 55,000 cells/mL in the period 15 to 45 days after calving. Approximately 20% of the first-parity cows had a SCC of ≥ 200,000 cells/mL in that study in the period 5 to 14 days after calving. More studies in this area are needed to establish an optimal cut-off value to distinguish between infected and non-infected first-parity cows.

Difficulties in handling a large number of variables in statistical modelling

In studies I-III a large number of variables were registered and collected from various databases resulting in hundreds of variables to analyse statistically. To analyse such a large number of variables is difficult due to confounding and risk of multicollinearity. To avoid this, the variables were first grouped into different categories (*e.g.* feeding, housing, milking), and then all variables were screen using univariable regression analysis. Variables that were significantly associated with the outcome (P < 0.20–0.25, depending on study) were then considered in multivariable regression analysis, one for each group of variables, provided collinearity between variables were < 0.7, and retained if $P \leq 0.10$. The final multivariable regression analysis model was then constructed using all variables retained in the multivariable analyses performed per group, retaining variables with significant association with the outcome of $P < 0.05$. Confounding was considered in all steps of the analyses. There are other methods to use, *e.g.* factor analysis and principal component analysis, to reduce the number of variables considered for analysis (Dohoo *et al.*, 1997). However, due to the risk of being subjective, and to difficulties in interpreting the results of these analysis techniques, we chose not to use them. Another possibility is to make indices or scores based on several input variables, but this makes it difficult to interpret the individual importance of the input variables. Another issue when dealing with a large number of variables is whether or not to remove variables showing a reverse causal relationship with the outcome of interest in the univariable analyses. We chose not to exclude such variables since these also can contribute information on the management/attitudes on a farm. However, other authors recommend excluding variables showing a relationship with the outcome that is reverse to biological assumptions, since it influences the results of other variables of more biological relevance (Bareille *et al.*, 2003).

Conclusions

The results from the studies presented in this thesis give insight into the epidemiology of mastitis in Swedish dairy herds, and it is concluded that:

- A high IRVTCM is not completely explained by a high incidence of CM, but also a reflection of the characteristics of the farmer and herd veterinarian. The farmer's attitude toward treatment of mastitis, and/or confidence in choosing when to treat a cow, need to be changed/supported in order to reduce excessive/unnecessary use of antimicrobials in our Swedish dairy herds.
- First-parity cows are at great risk of udder disorders in early lactation, as they were shown to have as high, or higher, incidence of VTCM in the period around calving and in early lactation than multiparous cows.
- Different factors are associated with different udder health measures, indicating that although efforts *e.g.* in reducing high SCC can reduce the risk of VTCM, this is not necessarily so.
- In Sweden, herds and cows of the SR-breed have, on average, better udder health than herds or cows of the SH-breed.
- Good hygienic conditions of the environment in the period around calving and good hygienic quality of the feed are factors of importance for udder health of first-parity cows.
- Metabolism and immune parameters in the period around calving are associated with SCC of first-parity cows in early lactation.
- Some metabolites and immune parameters differ between the SR- and SH-breeds, as well as between heifers of different age at calving.

Implications for future research

Much research has been performed in the area of bovine mastitis, but still mastitis is the most common disease of dairy cows. Udder health would most certainly improve if all research results were applied. One major issue is, however, how to communicate results to farmers so they are implemented. However, as dairy farming, including housing system, herd size and genetic material, undergoes constant changes further research on mastitis is needed.

Improved measures of udder health, and registration of CM

Investigations on finding optimal cut-off levels of SCC as indication of subclinical mastitis in first-parity cows are needed. Furthermore, ways to promote farmer/veterinarian registration of cases of CM should be investigated in order to improve health records and their use in mastitis research.

Attitudes of farmers and herd veterinarians, and on how to improve communication/application of research results

The attitude of the farmers was shown to be closely related to udder health at herd level in study I. More research on the nature of farmer attitudes and on the attitudes of veterinarians towards mastitis treatment could reduce excessive use of antibiotics. Moreover, the most efficient ways to spread the results from research to farmers, advisors and veterinarians should be investigated as there is a lot of easily applicable results on how to reduce the incidence of mastitis that does not seem to reach the farms.

The breed effect

Several differences between the SR- and SH-breeds are described in this thesis, but this is an area where more research is needed. Detailed studies on how the metabolic status of a cow influences the immune system should be performed. In addition, investigations on how feeding regimes and management influences the metabolism and immune system of cows of different breed could aid in improving feeding strategies in the period around calving.

The importance of the hygienic quality of feed

The results of the studies presented in this thesis imply that the hygienic quality of feed is important for udder health. However, this area needs to be investigated in detail to clarify its relationship with udder health, and which quality parameters that is most important. To get a better indication of the hygienic quality of feed frequent feed samples must be taken in each herd. The hygienic quality of corn silage and sugar-beet pulp needs special attention.

Concentrate feeding before and after calving

Studies I-III indicated that the amount of concentrates given before and after calving was of importance for the udder health of first-parity cows. However, the results were somewhat contradictory, and studies focusing on feed type, feed characteristics, interaction between nutrients and the physiological state of the animal, with more detailed recording of feeding are therefore needed to clarify associations between concentrates and udder health.

Comparisons of feeding and management of first-parity cow vs. older cows

It is important to investigate if different factors in housing, feeding, milking and management have the same impact on the udder health of first-parity cows as on the udder health of multiparous cows. As of now, most studies have looked at the whole herd, or only at the first-parity or multiparous cows in a herd. The results could be very useful in decisions on special efforts needed for each group of animals.

Measurements of stress

The changes in the period around calving in housing, feeding and management could be stressful for the cow and could negatively influence the immune system. Investigations on how to measure if these changes are stressful or not, perhaps by registering alterations of the behaviour of the cow, are needed.

Practical advice to dairy farmers

- Monitor first-parity cows closely in the month before and after calving, and keep them in a clean environment before, at, and after calving. It is preferable to let them calve alone. If group calving is necessary first-parity cows should only be grouped with other first-parity cows.
- The calving area should be cleaned thoroughly before a calving, and the cow should be moved from the area within a day or two after calving.
- If it is difficult to keep good hygiene at milking (cleaning, post-milking teat disinfectant), and/or the function/cleaning of the milking equipment at the calving site is inferior, milking at the normal milking site is preferable.
- If the cow is on pasture she should be housed in good time before calving, or wait at least one day after calving before housing to avoid excessive stress close to calving.
- Avoid giving inferior looking/smelling feed to the cows. If possible, cows avoid eating such feed, which reduces their nutrient intake. If the feed is infected with moulds, mycotoxins can adversely affect the immune system and udder health of the cows.
- If feed fed to first-parity cows are high in energy and low in protein, such as corn silage and/or sugar-beet pulp, make sure that the protein needs also are covered in the diet.
- Supplementation with vitamin E in the period around calving can be beneficial for the udder health
- If a cow has a reproductive disorder at calving (retained placenta, dystocia *etc*), preventive measures such as good bedding hygiene and strict hygiene at milking, would most likely protect some of those cows from also getting CM in this period.
- Choose cows of the SR-breed if you want to have cows with healthy udders!
- Excessive treatment with antimicrobials during lactation and at drying off will not remove the udder infections from the herd, but preventive measures will.

Populärvetenskaplig sammanfattning

Mastit (juverinflammation) är den vanligast förekommande sjukdomen hos våra mjölkkor och majoriteten av antibiotikabehandlingarna av mjölkkor görs i samband med mastit. Mastit klassas som subklinisk då inflammationen endast kan upptäckas genom att man i mjölken kan registrera ett ökat celltal (pga en ökning av antalet vita blodkroppar), eller klinisk då man kan se förändringar i mjölken (t.ex. flockor) ofta i samband med ett ömt och svullet juver och även ibland försämrat allmäntillstånd och feber.

Trots att juverhälsan, mätt i tankmjölkcelltal, i Sverige förbättrats under de senaste årtiondena har antalet kliniska mastiter legat relativt oförändrat. Forskning har dessutom visat att förstakalvare är minst lika mycket drabbade av juverhälsostörningar i tidig laktation som äldre kor. Man har också visat att kor som drabbas av klinisk mastit eller som har höga celltal i tidig laktation löper en högre risk för återkommande mastiter, minskad mjölkavkastning och kvarstående höga celltal under laktationen. En förklaring till att förstakalvarna drabbas så ofta i tidig laktation kan vara den stora omställningen i samband med kalvning. Kor som kalvar för första gången kommer oftast från en annan inhysning, har utfodrats annorlunda och har aldrig tidigare producerat mjölk eller mjölkats. Något som också skiljer förstakalvarna från äldre kor är att de förutom behovet av energi, protein och andra näringsämnen för underhåll och mjölkproduktion, även behöver näring för tillväxt då de fortfarande växer.

Syftet med denna avhandling var att finna faktorer, både på besättnings- och konivå, som påverkar risken för mastit, för att förbättra juverhälsoläget och minska onödig/överdriven antibiotikaanvändning i svenska mjölkkobesättningar.

För att uppnå avhandlingens syfte utfördes fyra olika studier, där totalt 343 kokontrollanslutna besättningar från framförallt södra Sverige, med totalt 3315 förstakalvare, deltog. Besättningarna valdes ut enligt olika kriterier. I studie I skulle besättningarna vara bland de kokontrollanslutna besättningar som hade högst mjölkavkastning och lägst förekomst subklinisk mastit, samt tillhöra den högsta femtedelen (HI-besättningar) eller lägsta fjärdedelen (LO-besättningar) med avseende på antalet veterinärbehandlade kliniska mastiter. Besättningarna, 158 st, följdes under ett år. I studie II valdes besättningarna ut efter deras inhysningssystem för kalvar och ungdjur, och förstakalvarna följdes från födsel till kalvning och under första laktationen. I studie III deltog 78 lösdriftsbesättningar med hög årlig mjölkproduktion och med lågt årligt tankmjölkscelltal. Av dessa 78 besättningar deltog 20 även i studie IV där betydelsen av ämnesomsättnings- och immunologiska parametrar för juverhälsan hos förstakalvare i perioden runt kalvning och i tidig laktation undersöktes.

Alla besättningar besöktes och lantbrukarna intervjuades om inhysningssystem, rengöringsrutiner, flytt av djur, bete, utfodring, mjölkning, förebyggande hälsoåtgärder, sinläggningsrutiner, tillvänjningsrutiner, behandlingskriterier m.m. I två av studierna togs även foderprov för analys av hygienisk kvalitet och i en studie bedömdes hygienen i foderlagringsutrymmen. Renhet, hull och tillväxt hos djuren bedömdes och registrerades i några av studierna. I studie I och III togs mjölkprov vid klinisk mastit för bakteriologisk undersökning. I studie IV togs blodprov från förstakalvare i perioden två veckor före till sex veckor efter kalvning för analys av selen, vitamin E, samt ett antal ämnesomsättnings- och immunologiska parametrar.

Olika juverhälsomått användes i de olika studierna för att undersöka de registrerade faktorernas inverkan på juverhälsan. I studie I jämfördes besättningar med hög förekomst av veterinärbehandlade kliniska mastiter med besättningar med låg förekomst. I studie II jämfördes förstakalvare som veterinärbehandlats för klinisk mastit i perioden -7 till 30 dagar efter kalvning med förstakalvare som inte veterinär behandlats för klinisk mastit i perioden. Dessutom jämfördes förstakalvare som hade celltal ≥ 200 000 celler/ml vid första provmjölkningen med förstakalvare som hade celltal < 200 000 celler/ml vid första provmjölkning. I studie III användes antal förstakalvare i besättningen som veterinärbehandlats för klinisk mastit i perioden -10 till 60 dagar efter kalvning, antal förstakalvare med celltal ≥ 200 000 celler/ml vid första provmjölkningen, samt förstakalvarnas celltal vid första provmjölkningen som juverhälsomått.

I studie IV undersöktes samband mellan analyserade blodparametrar och celltal vid första provmjölkning, samt om det fanns skillnader i de analyserade blodparametrarna mellan våra två vanligaste svenska mjölkraser (Svensk röd och vit boskap (SRB) och Svensk Holstein (SH)) och mellan förstakalvare med olika inkalvningsålder.

För att analysera sambandet mellan de registrerade faktorerna och juverhälsomåtten användes linjär, logistisk och Poisson regression. Totalt analyserades ca 650 faktorer och deras samband med juverhälsa, varav 41 faktorer fanns ha signifikanta samband med någon eller några av de sju juverhälsomåtten.

Resultaten från studie I visade att lantbrukare i HI-besättningar var mer benägna att antibiotikabehandla kor med höga celltal under laktationen och kor vid sinläggning, samt att kalla på veterinär för behandling av kor med klinisk mastit vid milda symptom. Det var vanligare att lantbrukare i LO-besättningar väntade tills korna visade tydliga symptom (feber, stört allmäntillstånd *etc.*) innan de kallade på veterinär. Dessa resultat tyder på att det föreligger olika behandlingsbenägenhet hos lantbrukarna. För att minska antibiotikaanvändningen i mjölkbesättningar bör lantbrukare och besättningsveterinärer informeras om vikten av förebyggande åtgärder.

Sammantaget tyder resultaten från de olika studierna på att det finns samband mellan foderhygien och juverhälsa, både på besättnings- och konivå. Ett foder med sämre hygienisk kvalitet kan innehålla mykotoxiner (gift producerat av mögelsvampar) som negativt inverkar på immunförsvaret och på så sätt ökar risken för mastit. Dessutom är ett foder med sämre hygien mindre smakligt och har sämre näringsinnehåll. Detta kan i sin tur leda till ökad risk för metaboliska störningar, som i sin tur är negativt för immunförsvaret och kan öka risken för juverhälsostörningar. Resultaten tyder även på att mängden kraftfoder som ges till kon i olika perioder kan påverka juverhälsan. Resultaten är emellertid något motsägelsefulla och studier där kraftfoderutfodringens påverkan på juverhälsan undersöks mer grundligt behövs.

Tre av studierna påvisade rasskillnader mellan kor av SRB-ras och SH-ras. Kor, eller besättningar med majoriteten av kor, av SRB-ras hade generellt ett bättre juverhälsoläge, än kor, eller besättningar med majoriteten av kor, av SH-ras. Den något lägre mjölkavkastningen hos SRB kan vara en del av förklaringen, men då raserna skiljde sig avseende de flesta analyserade ämnesomsättnings- och immunologiska parametrarna, kan man även spekulera i att SRB-kor har en stabilare ämnesomsättning och bättre immunförsvar. Mer forskning behövs dock för att bekräfta detta.

Studierna visade att förstakalvare har en hög förekomst av kliniska mastiter i tidig laktation och i studie I var förekomsten av kliniska mastiter minst lika hög som hos de äldre korna. Resultaten från studie II och III talar för vikten av hygien och tillvänjning i perioden runt kalvning. För att minska risken att en förstakalvare drabbas av juverhälsostörningar i tidig laktation bör man låta förstakalvare kalva ensamma och inte i grupp (ffa inte med äldre kor som kan sprida smitta), flytta henne från kalvningsutrymmet inom 1 dygn och inte mjölka henne i kalvningsutrymmet. Man bör heller inte stalla in förstakalvare som är på bete samma dag som kalvning, utan göra det i god tid före eller någon till några dagar efter kalvning för att minska stress. Vissa tvångsredskap vid mjölkning kan vara stressande och negativt påverka mjölknedsläpp, immunförsvar och juverhälsa. Resultaten visade även att förstakalvare som drabbas av förlossningsrelaterade störningar (t.ex. kvarbliven efterbörd, livmoderinflammation) i perioden runt kalvning har ökad risk för juverhälsostörningar. Kan man förebygga dessa förlossningsrelaterade störningar, med t.ex. tillskottsutfodring av vitamin E och selen, kan även förekomst av juverhälsostörningar minskas. Extra tid för skötsel och övervakning av förstakalvarna i perioden runt kalvning och i tidig laktation kan förbättra juverhälsoläget för dessa djur, och därmed också gynna mjölkproduktionen och mjölkkvaliteten.

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