

Mastitis in Dairy Cows

Genotypes, Spread, and Infection Outcome
of Three Important Udder Pathogens

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Mastitis in Dairy Cows. Genotypes, Spread, and Infection Outcome of Three Important Udder Pathogens

Abstract

Mastitis, inflammation of the udder, is a common disease among dairy cows worldwide. This thesis investigated the genotype variation and spread of three major udder pathogens: *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*. Isolates collected in a previous study of veterinary-treated clinical mastitis (VTCM) were used to study between-herd genotype variation in epidemiologically independent isolates and differences in outcome. Intramammary infections (IMI) were scrutinized for their occurrence on the day of calving and four days later by quarter milk sampling in selected herds with mastitis problems. The importance for long-term udder health and production of these IMI was also investigated.

The two most common *Staph. aureus* genotypes among the VTCM isolates were detected in 64% of the herds. In contrast, none of almost 100 *Strep. uberis* isolates from different herds was of the same genotype. The *Strep. dysgalactiae* isolates varied moderately compared to the ones of *Staph. aureus* and *Strep. uberis*. The common genotypes of *Staph. aureus* were associated with a lower somatic cell count (SCC) during the follow-up period, compared to the less common genotypes. No differences were detected between genotypes of streptococci, but cows with *Strep. dysgalactiae* VTCM had a lower SCC during the follow-up period compared to those with *Strep. uberis*. In herds with mastitis problems, *Staph. aureus* was the most common pathogen found at and just after calving, followed by *Strep. dysgalactiae*, and *Strep. uberis*. Isolates of *Staph. aureus* showed the lowest within-herd genotype diversity, followed by an intermediate diversity of *Strep. dysgalactiae* and a high diversity of *Strep. uberis*. There was a marked variation in occurrence of IMI at or close to calving in herds with mastitis problems, indicating that the predisposing factors for udder infections at calving differed between herds. Most early lactation IMI were associated with an increase in lactation SCC, whereas associations with other outcome variables were more variable. Altogether, this thesis contributes knowledge about *Staph. aureus*, *Strep. dysgalactiae*, and *Strep. uberis* that can be used in preventive work against these IMI.

Keywords: *Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, clinical mastitis, intramammary infection, outcome, bacterial genotype, early lactation

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Dedication

To my family and friends

living is easy with eyes closed, misunderstanding all you see

John Lennon

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List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I Lundberg, Å., Aspán, A., Nyman, A., Ericsson Unnerstad, H., & Persson Waller, K. (2014). Associations between bacterial genotype and outcome of bovine clinical *Staphylococcus aureus* mastitis. *Acta Veterinaria Scandinavica* 56(2), 1-8.
- II Lundberg, Å., Nyman, A., Ericsson Unnerstad, H., & Persson Waller, K. (2014). Prevalence of bacterial genotypes and outcome of bovine clinical mastitis due to *Streptococcus dysgalactiae* and *Streptococcus uberis*. *Acta Veterinaria Scandinavica* 56(80), 1-11.
- III Lundberg, Å., Nyman, A-K., Aspán, A., Börjesson, S., Ericsson Unnerstad, H., & Persson Waller, K. Udder infections with *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus uberis* at calving in dairy herds with mastitis problems. (Submitted for publication)
- IV Lundberg, Å., Nyman, A-K., & Persson Waller, K. Long-term effects of udder infections with *Staphylococcus aureus*, *Streptococcus dysgalactiae* and *Streptococcus uberis* at calving in dairy herds with mastitis problems. (Manuscript).

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The contribution of Åsa Lundberg to the papers included in this thesis was as follows:

- I Mainly responsible for performing genotyping. Analysed the results in collaboration with the supervisors. Performed the statistical analyses under supervision and wrote the manuscript with regular input from the co-authors.
- II Was involved in the initial genotyping. Was responsible for analyses of the results and performed the statistical analyses under supervision. Was responsible for writing and completing the manuscript with regular input from the co-authors.
- III Was involved in planning of the practical study. Was responsible for recruitment of herds and visited the herds. Contributed to laboratory work (mainly initial assessment of milk sample results). Was responsible for analyses of all results including those of genotyping. Performed the statistical analyses under supervision. Was responsible for writing and completing the manuscript with regular input from the co-authors.
- IV Participated in the design of the project. Performed statistical analyses in collaboration with the supervisors. Was responsible for writing the manuscript with regular input from the co-authors.

Abbreviations

CC	Clonal complex
CLE	Cleared infection, pathogen only present at the D0 sampling
CM	Clinical mastitis
D0	The day of calving
D4	Day four after calving
DIM	Days in milk
IMI	Intramammary infection/infections
NEG	Negative, no findings of pathogens at any sampling
NEW	New infection, pathogen only present at the D4 sampling
PER	Persistent infection, pathogen present at both samplings
PFGE	Pulsed-field gel electrophoresis
SADRS	Swedish Animal Disease Recording System
SCC	Somatic cell count
SCM	Subclinical mastitis
SH	Swedish Holstein breed
SOMRS	Swedish Official Milk Recording Scheme
<i>spa</i>	<i>Staphylococcus</i> protein A gene
SR	Swedish Red breed
<i>Staph.</i>	<i>Staphylococcus</i>
<i>Strep.</i>	<i>Streptococcus</i>
VTCM	Veterinary-treated clinical mastitis

1 Introduction

Mastitis means inflammation of the udder and is a common disease among dairy cows worldwide. It is often associated with bacterial intramammary infections (IMI) and is subdivided into clinical mastitis (inflammation with visual signs of inflammation in the udder or milk; CM) and subclinical mastitis (inflammation without visual signs; SCM). Both CM and SCM influence milk quality and yield negatively, and mastitis is therefore of major economic concern for the farmer. Clinical mastitis is also of potential concern from an animal welfare perspective.

The dairy sector in Sweden is undergoing major changes. The number of farms is continuously decreasing, and the average number of cows per farm continues to increase. In addition, a switch from tie stalls to free-stall housing is in progress. As management systems influence spread of udder pathogens, the ongoing changes in the dairy sector result in new challenges for farmers, as well as for veterinarians, other advisers, and researchers. Therefore, research providing up-to-date knowledge about infection patterns within and between herds of the most common udder pathogens is warranted.

This thesis investigates genotype variation and spread of *Staphylococcus (Staph.) aureus*, *Streptococcus (Strep.) dysgalactiae*, and *Strep. uberis*, three common udder pathogens causing mastitis in dairy cows in Sweden. In addition, the short- and long-term outcomes of these infections are investigated.

1.1 The dairy cow sector in Sweden

In 2013, there were around 344 000 dairy cows in 4 668 herds in Sweden, giving an average herd size of 74 cows per herd (Swedish Board of Agriculture, 2014). The number of dairy cows has decreased 48% since 1980 and the number of herds has decreased 89% (Swedish Board of Agriculture,

2014). Eighty-five percent of the dairy cows are in the southern third of Sweden (Swedish Board of Agriculture, 2014).

About 84% of Swedish dairy cows are enrolled in the Swedish Official Milk Recording Scheme (SOMRS, Växa Sverige; Växa Sverige, 2014a). Free-stall housing is used in about 40% of enrolled farms and these farms housed 60% of the cows in 2013/2014. Major breeds are Swedish Red (SR, 39.5%) and Swedish Holstein (SH, 52.9%; Växa Sverige, 2014a). Average milk yield in 2012/2013 from the enrolled cows was 9 200 kg per cow and year, which can be compared to an average annual milk yield in 1980 of 5 900 kg per cow (Växa Sverige, 2014a).

Among dairy cows enrolled in the SOMRS, the most common veterinary-treated diagnoses in 2012/2013 were mastitis (14.3 cases per 100 cow-years) and puerperal paresis (3.1 cases per 100 cow-years; Växa Sverige, 2014a). Swedish herds are free of epizootic diseases such as bovine viral diarrhoea virus (BVDV; Växa Sverige, 2014b), enzootic bovine leucosis, and bovine tuberculosis (Anonymous, 2013).

The average age at culling for a Swedish dairy cow enrolled in the SOMRS was 60.5 months in 2012/2013; the major culling reasons were decreased fertility (21.7%), mastitis (14.9%), low milk yield (10.7%), chronic udder health problems (7.8%), and claw and leg disorders (7.7%; Växa Sverige, 2014a).

1.2 General aspects of mastitis

Mastitis is categorized as acute or chronic on the basis of duration and, as mentioned above, it is also divided into CM and SCM on the basis of symptoms. Clinical mastitis is classified by the types of symptoms: mild (clotting of milk), moderate (changes in milk and visible signs of inflammation of the udder), or severe (changes in milk and udder, and systemic signs).

The milk somatic cell count (SCC) measures concentration of somatic cells, predominantly inflammatory ones, and is an important diagnostic tool for SCM. The SCC from a healthy udder is about 70,000 cells/ml (Djabri *et al.*, 2002) depending on age, breed, stage of lactation, and milk yield of the cow (Emanuelson & Funke, 1991; Schepers *et al.*, 1997; Nyman *et al.*, 2014). A threshold of 200 000 cells/ml has been proposed and is often used to distinguish SCM from healthy udders (Dohoo & Leslie, 1991), but lower (Halasa *et al.*, 2009; Madouasse *et al.*, 2010; Archer *et al.*, 2014) or higher thresholds are used in some studies (Pitkälä *et al.*, 2004; Deluyker *et al.*, 2005).

Intramammary infections are the most common cause of mastitis. These are defined as infections of the mammary gland secretory tissue and/or of the ducts

and tubules by pathogens (International Dairy Federation, 2011). Many micro-organisms can infect the udder, but bacteria, especially staphylococci, streptococci, and coliforms are the most common pathogens associated with mastitis. In Sweden, *Staph. aureus*, *Strep. dysgalactiae*, and *Strep. uberis* are three of the most common udder pathogens, accounting for 21, 16, and 11% of veterinary-treated CM (VTCM), respectively (Ericsson Unnerstad *et al.*, 2009), and 19, 9, and 8% of subclinical cases (Persson *et al.*, 2011). The prevalence of pathogens associated with VTCM has been relatively stable the last 30 years in Sweden (Funke, 1983; Ericsson Unnerstad *et al.*, 2009).

1.3 Occurrence of mastitis and IMI

Reports on mastitis focus on CM, SCM, and/or IMI and as definitions used may vary, comparisons of different studies have to be made with care. Furthermore, the incidence and prevalence varies between countries and regions, and may be affected by factors such as parity, stage of lactation, season, and herd.

1.3.1 General aspects

The Swedish incidence rate of CM is estimated to 26 cases per 100 cow-years, and corresponding numbers for other countries range from 23 to 65 (Bradley *et al.*, 2007; Olde Riekerink *et al.*, 2008; Wolff *et al.*, 2012). The prevalence of SCM in Sweden is about 35 cases per 100 cow-years (Växa Sverige, 2013), and the corresponding prevalence in Finland was 31 in 2001 (Pitkälä *et al.*, 2004).

The occurrence of CM and SCM increases with increasing parity (Barkema *et al.*, 1998; Hagnestam *et al.*, 2007; Olde Riekerink *et al.*, 2007; Persson Waller *et al.*, 2009; Verbeke *et al.*, 2014). In Sweden, the incidence of VTCM in 2012/2013 increased from 7.1 per 100 cow-years for primiparous cows to 23.1 in sixth and higher parities (Växa Sverige, 2013). However, in some studies incidence rate of CM in primiparous cows is as high as in considerably older cows (McDougall *et al.*, 2007b).

The incidence of SCM increases with days in milk (DIM; Busato *et al.*, 2000; Olde Riekerink *et al.*, 2007; Abrahmsén *et al.*, 2014), but cows are at the highest risk for CM around calving and in early lactation (Valde *et al.*, 2004; Svensson *et al.*, 2006; McDougall *et al.*, 2007b; Olde Riekerink *et al.*, 2007, 2008; Persson Waller *et al.*, 2009; Verbeke *et al.*, 2014) and many cows have high milk SCC at first test milking after calving (Svensson *et al.*, 2006; Madouasse *et al.*, 2010; Archer *et al.*, 2014). Primiparous cows are especially exposed during early lactation and most cases of CM in primiparous cows

occur within one or two weeks after calving (Olde Riekerink *et al.*, 2008; Persson Waller *et al.*, 2009). As this is a period when the primiparous udder is still developing, inflammatory reactions at this time can be detrimental for future udder health and production.

Occurrence of mastitis and IMI varies with season. Olde Riekerink *et al.* (2007) reported a peak incidence rate of CM in winter but a peak in bulk tank SCC in August to September. In Finland, the frequency of heifer CM was highest in late spring and in late summer (Myllys & Rautala, 1995). In addition, the relative importance of different udder pathogens varies throughout the year (Waage *et al.*, 1999; Østerås *et al.*, 2006; Olde Riekerink *et al.*, 2007; Ericsson Unnerstad *et al.*, 2009).

The occurrence of CM, SCM, and IMI, and of predominant udder pathogens, varies markedly between herds, probably due to differences in housing systems, hygiene, and management (Myllys & Rautala, 1995; Barkema *et al.*, 1998; Olde Riekerink *et al.*, 2008; Tenhagen *et al.*, 2009; Verbeke *et al.*, 2014).

1.3.2 Specific pathogens

Staphylococcus aureus

Staphylococcus aureus is an important udder pathogen in Sweden, Belgium, Canada, Ireland, and Norway, as well as in other countries (Østerås *et al.*, 2006; Olde Riekerink *et al.*, 2008; Ericsson Unnerstad *et al.*, 2009; Persson *et al.*, 2011; Keane *et al.*, 2013; Verbeke *et al.*, 2014), but the relative importance of the pathogen has decreased during the last decades in some countries and regions including USA, England, and Finland (Wilson & Richards, 1980; Makovec & Ruegg, 2003; Bradley *et al.*, 2007; Ferguson *et al.*, 2007; Sampimon *et al.*, 2009).

Staphylococcus aureus can be a common cause of CM in both primiparous and multiparous cows (Waage *et al.*, 1999; McDougall *et al.*, 2007b; Persson Waller *et al.*, 2009) and *Staph. aureus* IMI have been found in heifers already before breeding, and in mid-to-late pregnancy (Trinidad *et al.*, 1990; Middleton *et al.*, 2005). However, a Danish study rarely found *Staph. aureus* IMI in heifers during the month before calving (Aarestrup & Jensen, 1997).

Peak incidence of *Staph. aureus* CM is reported to occur in early lactation for primiparous cows (Olde Riekerink *et al.*, 2007; Persson Waller *et al.*, 2009), and a decrease in *Staph. aureus* IMI with an increase in DIM, regardless of parity, has also been reported (Østerås *et al.*, 2006).

An increase in the occurrence of *Staph. aureus* CM in late autumn or winter is reported in several studies (Waage *et al.*, 1999; Olde Riekerink *et al.*, 2007;

Ericsson Unnerstad *et al.*, 2009). However, a small peak in CM during the summer and a high IMI prevalence in June to July have also been noted (Østerås *et al.*, 2006; Olde Riekerink *et al.*, 2007), and no seasonality in prevalence of *Staph. aureus* IMI was detected in Sicily, Italy (Ferguson *et al.*, 2007).

Staphylococcus aureus prevalence varies between herds (Barkema *et al.*, 1998; McDougall *et al.*, 2007b; Tenhagen *et al.*, 2009), and the pathogen is reported to be more common in tie-stall housing systems compared to other housing systems (Olde Riekerink *et al.*, 2008; Ericsson Unnerstad *et al.*, 2009).

Streptococcus dysgalactiae

Streptococcus dysgalactiae subsp. *dysgalactiae* is a common udder pathogen in Sweden, Norway, parts of Canada, and the Netherlands (Barkema *et al.*, 1998; Østerås *et al.*, 2006; Olde Riekerink *et al.*, 2008; Ericsson Unnerstad *et al.*, 2009; Persson *et al.*, 2011) but in other areas of the world, such as England and Wales, Germany, and Uruguay, the pathogen contributes to only small proportions of CM and SCM (Giannechini *et al.*, 2002; Tenhagen *et al.*, 2006; Bradley *et al.*, 2007).

Streptococcus dysgalactiae IMI have been reported to increase with parity and DIM (Østerås *et al.*, 2006; Sampimon *et al.*, 2009). However, *Strep. dysgalactiae* IMI is also a relatively common finding in heifers during the week before calving (Aarestrup & Jensen, 1997). These prepartum IMI often persist past calving, but prevalence decreases in the second week of lactation (Aarestrup & Jensen, 1997). In Sweden, the pathogen is a common cause of CM in early lactation primiparous cows (Persson Waller *et al.*, 2009).

In Norway, the proportions of *Strep. dysgalactiae* IMI and of heifer CM were highest in the late indoors season (Waage *et al.*, 1999; Østerås *et al.*, 2006), and in another study the incidence rate of *Strep. dysgalactiae* CM peaked in January (Olde Riekerink *et al.*, 2007). No seasonal trend was identified in prevalence of *Strep. dysgalactiae* VTCM in Sweden (Ericsson Unnerstad *et al.*, 2009).

Streptococcus uberis

Streptococcus uberis is a common udder pathogen in Sweden and worldwide (Bradley *et al.*, 2007; McDougall *et al.*, 2007b; Verbeke *et al.*, 2014). It is especially common in pasture-based systems (Compton *et al.*, 2007; McDougall *et al.*, 2007b) and in management systems with large free-stall herds (Bradley *et al.*, 2007). In many parts of the world, its relative significance has increased during the last decades, whereas the significance of

other pathogens such as *Staph. aureus* and *Strep. agalactiae* has decreased, as reviewed by Ruegg (2012).

The prevalence of *Strep. uberis* IMI increases with parity according to a few studies (Zadoks *et al.*, 2001b; Sampimon *et al.*, 2009). McDougall *et al.* (2007b) reported that the relative CM prevalence of the pathogen declines with DIM while Zadoks *et al.* (2001b) reported that the association between *Strep. uberis* prevalence and stage of lactation was herd dependent.

Occurrence of *Strep. uberis* is reported to peak in summer and is associated with pasture season in non-pasture based systems (Østerås *et al.*, 2006; Olde Riekerink *et al.*, 2007). In Sweden, a lower proportion of *Strep. uberis* CM was noted in January to April compared to September to December (Ericsson Unnerstad *et al.*, 2009).

Between-herd variation in *Strep. uberis* prevalence has been reported (Østerås *et al.*, 2006; McDougall *et al.*, 2007b), and the occurrence of *Strep. uberis* IMI and CM is higher in tie-stall barns compared to other systems according to a few studies (Ferguson *et al.*, 2007; Olde Riekerink *et al.*, 2008).

1.4 Genetic variation and spread of infection

1.4.1 General aspects

Udder pathogens are often divided into contagious and environmental on the basis of their main reservoirs. Contagious pathogens are well adapted to the cow udder and spread primarily from infected to uninfected mammary glands at milking (International Dairy Federation, 2011), for example by milking machines and milkers' hands (Fox & Gay, 1993). In contrast, environmental pathogens readily colonize and multiply in the environment (Bramley & Dodd, 1984; Smith & Hogan, 1993) and are usually transferred to the udder from these sources, although transmission from other udders via the milking machine is also possible.

The genetic variation of contagious pathogens is limited, whereas the variation of environmental pathogens is substantial (Tenover *et al.*, 1995; Wang *et al.*, 1999). Genotyping of udder pathogens can therefore contribute to understanding the spread of udder infections.

Various genotyping methods for *Staph. aureus*, *Strep. dysgalactiae*, and *Strep. uberis* have been used and evaluated. The method chosen for a particular purpose is based on the level of discriminatory power needed, as well as on time and economic considerations, laboratory resources, and need for inter-laboratory comparisons. Pulsed-field gel electrophoresis (PFGE) was introduced for genotyping of udder pathogens in the mid-1990s (Bannerman *et al.*, 1995). For many years it was the gold standard in bacteriology for research

purposes because of excellent typeability, discriminatory power, and easy interpretation (Olive & Bean, 1999; Zadoks *et al.*, 2002; Hallin *et al.*, 2007). This method generates macro-restriction patterns by enzymatic cleavage of the bacterial genome. The generated patterns can be compared manually or by software. Identical banding patterns are considered to be of the same pulsotype, while banding patterns with up to a three-band difference or of a similarity of above about 80% are usually grouped into clusters or lineages as probably genetically related.

spa typing involves sequencing of the polymorphic X region of the protein A gene (*spa*) of *Staph. aureus* (Frénay *et al.*, 1996). The method is a common method for genotyping methicillin resistant *Staph. aureus* and has become increasingly used in *Staph. aureus* genotyping studies of bovine isolates (Aires-de-Sousa *et al.*, 2007; Hata *et al.*, 2010; Bar-Gal *et al.*, 2015). It is relatively cheap and less time-consuming than PFGE, and has a discriminatory power at about the same level as PFGE clusters/lineages. It is also an excellent method for comparing strains from different herds, regions and countries, for outbreaks and surveillance (Cookson *et al.*, 2007).

1.4.2 Specific pathogens

Staphylococcus aureus

Staphylococcus aureus IMI can be caused by a large variety of bacterial genotypes (Zadoks *et al.*, 2000; Buzzola *et al.*, 2001; Sabour *et al.*, 2004; Tenhagen *et al.*, 2007; Fournier *et al.*, 2008; Capurro *et al.*, 2010a). However, one or a few genotypes are often more widespread than others both within and between herds (Sabour *et al.*, 2004; Mørk *et al.*, 2005; Smith *et al.*, 2005; Fournier *et al.*, 2008; Capurro *et al.*, 2010a). The pathogen is categorized as contagious. However, *Staph. aureus* can also be cultured from animal body sites, in particular hock skin, as well as from heifers and from the close environment of the cow, suggesting that there are environmental sources as well (Matos *et al.*, 1991; Roberson *et al.*, 1994, 1998; Capurro *et al.*, 2010b; Anderson *et al.*, 2012). Body site isolates and environment isolates can be of the same genotype as milk isolates from IMI (Gillespie *et al.*, 1999; Zadoks *et al.*, 2002; Haveri *et al.*, 2008; Capurro *et al.*, 2010b; Anderson *et al.*, 2012), but the importance of sources outside the udder is still unclear.

The genetic variation of *Staph. aureus* in Sweden has been investigated using a limited collection of isolates from defined parts of the country (Capurro *et al.*, 2010a). A large number of genotypes could be identified, but three genotypes were widespread in Sweden, although one of them was mostly restricted to the southern part of Sweden (Capurro *et al.*, 2010a). Sources of

Staph. aureus in a few tie-stall herds have also been studied in Sweden (Capurro *et al.*, 2010b), but corresponding knowledge of infection patterns and sources of infections in the peripartum period in free-stall herds is missing.

Streptococcus dysgalactiae

In North American studies, it has been uncommon to type streptococci to species level, except for *Strep. agalactiae*; instead these bacteria have been included in the group environmental streptococci (Smith & Hogan, 1993; Todhunter *et al.*, 1995). In other studies, however, *Strep. dysgalactiae* is categorized as a contagious pathogen (Bramley & Dodd, 1984; Fox & Gay, 1993; Barkema *et al.*, 1999).

Streptococcus dysgalactiae is one of the pathogens associated with summer mastitis (Madsen *et al.*, 1990) of heifers and dry cows, and it has been shown that the fly *Hydrotaea irritans* can act as a vector for *Strep. dysgalactiae* (Chirico *et al.*, 1997). The pathogen has also been isolated from extra-mammary body sites (Cruz Colque *et al.*, 1993; Calvinho *et al.*, 1998), although information about these sources is scarce.

The number of genotyping studies of *Strep. dysgalactiae* is rather low (Baseggio *et al.*, 1997; Gillespie *et al.*, 1998; Wang *et al.*, 1999). A number of genotypes are usually present within a herd, but one genotype is often found in multiple cows (Baseggio *et al.*, 1997; Wang *et al.*, 1999), which indicates that *Strep. dysgalactiae* can be spread from cow to cow. In addition, genetically related isolates have been found on multiple farms, suggesting either contagious spread between cows and herds or a common environmental source (Baseggio *et al.*, 1997; Wang *et al.*, 1999). However, there are no studies of genotype prevalence in a national survey material, and the genotype variation in Swedish *Strep. dysgalactiae* isolates remains unknown.

Streptococcus uberis

Streptococcus uberis is considered an environmental pathogen and in North American studies the pathogen is often grouped as environmental streptococci with *Strep. dysgalactiae*, instead of being typed to species level. It has been cultured from a number of sources outside the udder, such as tonsils, water, soil, bedding material, flies, faeces, and farm tracks (Cruz Colque *et al.*, 1993; Zadoks *et al.*, 2005; Lopez-Benavides *et al.*, 2007).

Molecular studies of *Strep. uberis* have confirmed that this pathogen predominantly shows a pattern consistent with environmentally spread bacteria (Wang *et al.*, 1999; McDougall *et al.*, 2004; Gilbert *et al.*, 2006; Lasagno *et al.*, 2011; Abureema *et al.*, 2014), although there is evidence that it also can spread

between cows as a contagious pathogen within a herd (Phuektes *et al.*, 2001; Zadoks *et al.*, 2001a, 2003; Coffey *et al.*, 2006).

The genotype variation of *Strep. uberis* in Sweden has not previously been investigated.

1.5 Treatment and outcome of mastitis or IMI

1.5.1 General aspects

Generally, mastitis treatment choices are made on the basis of bacteriological culture and antimicrobial susceptibility testing when applicable. The choice of treatment is also made from clinical manifestation and prognosis, and depends on legislation and available drugs. The prognosis after treatment is defined by pathogen, antimicrobial susceptibility, chronicity of infection, infection load, age of the cow, breed, and number of quarters affected (Sol *et al.*, 1994; Owens *et al.*, 1997; Østerås *et al.*, 1999; Sol *et al.*, 2000; Deluyker *et al.*, 2005; Sandgren *et al.*, 2008).

In Sweden, bacteriological culturing is recommended before initiation of antimicrobial treatment of CM. All veterinary treatments should be reported by the veterinarian to the Swedish Animal Disease Recording system (SADRS; Swedish board of agriculture, Jönköping, Sweden), and antimicrobials can only be prescribed by a veterinarian. The first choice of antimicrobial treatment of gram-positive pathogens in Sweden is intramuscular administration of benzyl penicillin (The Swedish Society of Veterinary Medicine (SVS), 2011) and mastitis was the reason for 69% of all parenteral treatments with antimicrobials to Swedish dairy cows in 2013/2014 (Växa Sverige, 2014b).

Antimicrobial treatment during lactation is not recommended for SCM; if these cases are to be treated, it is done at drying-off. Selective dry cow therapy based on udder health parameters of the individual cow is practiced in Sweden.

Outcome after mastitis or IMI can be measured as bacteriological or clinical (if CM) cure during a follow-up period. It can also be measured in terms of udder health parameters such as SCC, new or additional cases of CM, and culling due to mastitis (De Vliegher *et al.*, 2004, 2005; Barkema *et al.*, 2006; Reksen *et al.*, 2006; Swinkels *et al.*, 2013). Production parameters (milk yield and milk components) are also widely used outcome parameters (Myllys & Rautala, 1995; Compton *et al.*, 2007; Archer *et al.*, 2013), as well as culling regardless of reason (Myllys & Rautala, 1995; De Vliegher *et al.*, 2005; Reksen *et al.*, 2006; Compton *et al.*, 2007).

In general, an increased SCC for a given time after mastitis is seen (de Haas *et al.*, 2002; De Vliegher *et al.*, 2004), as is a decrease in milk yield (Myllys & Rautala, 1995; Gröhn *et al.*, 2004; Hagnestam *et al.*, 2007; Halasa *et al.*, 2009).

In addition, both CM and SCM are associated with an increased risk of new CM cases and culling (Myllys & Rautala, 1995; De Vliegher *et al.*, 2005; Compton *et al.*, 2007). As stated above, outcome of mastitis and IMI is predicted by host factors, but it is also pathogen-dependent (Paradis *et al.*, 2010; Piepers *et al.*, 2010; Pearson *et al.*, 2013) and can be strain-specific (Haveri *et al.*, 2005). It is of special interest to identify IMI important for long-term udder health, as these cases would be the most economically favourable to treat and/or prevent.

1.5.2 Specific pathogens

Staphylococcus aureus

Despite the use of antimicrobials chosen according to susceptibility testing, cure rates of *Staph. aureus* CM are often low (Pyörälä & Pyörälä, 1998; Sol *et al.*, 2000). In addition, as low as 1-30% of *Staph. aureus* SCM are cured spontaneously (Sandgren *et al.*, 2008), and bacteriological cure after treatment of SCM is variable but generally low (Wilson *et al.*, 1999; Sandgren *et al.*, 2008). The highest cure rates of CM and SCM are associated with lower parity, shorter duration of IMI, a lower number of udder quarters infected, and infections with non- β -lactamase producing strains (Sol *et al.*, 2000; Taponen *et al.*, 2003b; Deluyker *et al.*, 2005). Breed differences in cure rate has also been reported (Sandgren *et al.*, 2008). After treatment of CM, the SCC can remain high throughout lactation in both primiparous and multiparous cows (de Haas *et al.*, 2002).

Intramammary infections with *Staph. aureus* in early lactation can negatively influence SCC and milk yield throughout lactation (Whist *et al.*, 2009; Paradis *et al.*, 2010), but one study demonstrated no effect on milk yield of *Staph. aureus* during the follow-up period (Paradis *et al.*, 2010).

Differences between *Staph. aureus* genotypes in severity of clinical signs, inflammatory response as measured by SCC, and persistence of infection have been observed (Fitzgerald *et al.*, 2000; Zadoks *et al.*, 2000; Haveri *et al.*, 2005; Fournier *et al.*, 2008). Moreover, the likelihood of cure after antimicrobial therapy may also differ between genotypes. Haveri *et al.* (2005) found that infection with one *Staph. aureus* pulsotype is related to severe symptoms, but the same pulsotype is also invariably eliminated from the udder after treatment with antimicrobials. In addition, Dingwell *et al.* (2006) found that some genotypes of *Staph. aureus* may be more likely to be eliminated by dry cow treatment. The outcome of Swedish *Staph. aureus* strains has not yet been investigated.

Streptococcus dysgalactiae

Bacteriological cure rate after *Strep. dysgalactiae* CM treated with benzyl penicillin or related compounds ranges from 65 to 90% in different studies (Taponen *et al.*, 2003a; McDougall *et al.*, 2007a; Kalmus *et al.*, 2014) and spontaneous cure after SCM ranged from 80% in first parity cows of the SR breed to 8% in cows of SH breed in third parity and higher (Sandgren *et al.*, 2008). Data on clinical cure rate after treatment of CM is scarce, but has been reported to 74% for *Strep. dysgalactiae* in one study (Kalmus *et al.*, 2014). In a study on treatment of heifers for CM caused by *Strep. dysgalactiae* around calving, 15% of the quarters were non-functional, and another 36% had an increased SCC in the milk and/or an IMI, 30 days after treatment (Waage *et al.*, 2000).

Increased SCC after CM has also been reported by De Haas *et al.* (2002), who described a slow decrease in SCC after VTCM in primiparous cows but an SCC that remained high throughout lactation for multiparous cows. Whist *et al.* (2007) demonstrated that early lactation IMI with *Strep. dysgalactiae* was associated with an increase in SCC, VTCM, and culling, and a decrease in milk yield throughout lactation.

Genotype specific clinical manifestation or outcome has not yet been investigated for *Strep. dysgalactiae* IMI using molecular methods, but in an experimental study of infections with four different *Strep. dysgalactiae* strains, strain specific pathogenicity was described (Higgs *et al.*, 1980).

Streptococcus uberis

Bacteriological cure rate of *Strept. uberis* CM treated with benzyl penicillin or related compounds has in field studies been reported to 45 to 92% (Taponen *et al.*, 2003a; McDougall *et al.*, 2007a; Kalmus *et al.*, 2014) and clinical cure rate was 77% in one study (Kalmus *et al.*, 2014). Spontaneous cure of SCM with *Strep. uberis* is slightly lower than that of *Strep. dysgalactiae*, with a range of 68% in first parity cows of the SR breed to 4% in cows of SH breed in third parity and higher (Sandgren *et al.*, 2008).

A decrease in milk yield in primiparous cows with *Strep. uberis* IMI at calving compared to a monozygous twin without IMI at calving has been described (Pearson *et al.*, 2013), but *Strep. uberis* IMI was not associated with an increase in SCC other than during the first month after calving in the same study. However, somewhat contradicting, De Haas *et al.* (2002) reported that a high SCC caused by *Strep. uberis* VTCM slowly decreased in primiparous cows over months, but remained high throughout lactation in multiparous cows.

Differences in virulence between *Strep. uberis* strains have been reported (Phuektes *et al.*, 2001; Zadoks *et al.*, 2003) and in an experimental study a difference in pathogenicity between a host-adapted strain and a non-adapted strain was demonstrated (Tassi *et al.*, 2013). However, knowledge about strain-specific virulence and outcome of *Strep. uberis* strains is scarce compared to that of *Staph. aureus*.

1.6 Prevention of mastitis

The best way to reduce prevalence of mastitis is to prevent new IMI. However, few intervention studies have been performed and preventive measures are generally suggested on the basis of risk factors associated with mastitis or specific pathogens rather than on the results of intervention studies.

Recommended preventive measures are usually based on the proposed 10 Point Plan (NMC, 2011) and depend on herd management system (i.e. conventional milking or robotic milking, tie stalls or free housing, etc.) and/or on the most prevalent udder pathogens present. Mastitis caused by contagious pathogens are mainly prevented through improvements in milking hygiene, use of post-milking teat disinfectants, blanket dry-cow therapy, and treatment, segregation, or culling of infected animals, while environmental pathogens are primarily prevented by improvement in barn or pasture hygiene and general optimization of the cows' immune system.

In Sweden, the most commonly recommended prevention strategies for *Staph. aureus* and *Strep. dysgalactiae* comprise post-milking teat disinfection, infectious disease control around calving, well-adjusted milking machines, and good infectious disease control at milking (Växa Sverige, 2015). Primary recommendations for *Strep. uberis* prevention comprises hygiene at milking and improvements in barn and pasture hygiene (Växa Sverige, 2015). However, as presented above, all three pathogens seem to be able to spread both in a contagious and in an environmental fashion complicating prevention, and some farms experience mastitis problems despite a perception of well-implemented preventive measures.

More knowledge about the impact of udder pathogens and bacterial genotype on udder health and production, as well as a better understanding of herd variation in IMI occurrence in regards to species, genotypes of species, seasonal variations, and parities is needed. With more knowledge about spread of infections, and more knowledge about which infections have the highest impact on udder health, the best prevention methods, motivated both economically and by animal welfare, can more easily be chosen.

2 Aims of the Study

The general aim of this thesis was to gather knowledge about udder infections caused by *Staph. aureus*, *Strep. dysgalactiae*, and *Strep. uberis* in Swedish dairy cows, with focus on genetic variation, spread, and infection outcome. With increased knowledge, improved prevention strategies can be designed in the future.

More specifically the aims were:

- To investigate the genetic variation of *Staph. aureus*, *Strep. dysgalactiae*, and *Strep. uberis* isolates collected from cases of CM within Sweden, and to investigate if genotype within species differ regarding spread between herds and disease outcome.
- To investigate the occurrence of *Staph. aureus*, *Strep. dysgalactiae*, and *Strep. uberis* IMI at and just after calving in large free-stall herds with mastitis problems, and to investigate if the infection patterns differed between bacterial species, herds, seasons, and parities.
- To investigate associations between *Staph. aureus*, *Strep. dysgalactiae*, or *Strep. uberis* IMI in early lactation, and udder health, production, and culling during the following lactation, and to investigate if the outcome varied depending on when IMI occurred in relation to calving.
- To investigate potential sources of *Staph. aureus* and *Strep. dysgalactiae* in body sites and the close environment of late-gestation heifers, dry cows, and in calving premises, in large free-stall herds with mastitis problems.

3 Materials and Methods

A summary of the materials and methods applied in papers I to IV is given below, as well as a detailed description of materials and methods in an additional study investigating potential body site and environmental sources of *Staph. aureus* and *Strep. dysgalactiae*. Other detailed descriptions are found in papers I to IV.

3.1 Inclusion of isolates, cows and herds

The studies in papers I and II used isolates from cases of VTCM collected in 2002/2003 in a national survey on the distribution of udder pathogens. For papers III and IV, 13 and 19 herds, respectively, were selected from which all primiparous and multiparous cows with even-numbered ear tags were sampled during a 12-month period starting in January 2011 and ending in March 2012.

All isolates in papers I and II were from herds enrolled in the SOMRS, as were all the herds in papers III and IV. For papers III and IV, additional inclusion criteria were: herd size (75-250 milking cows per year), main breed of the herd (at least 75% of SH, SR, or SHxSR crossbreeds), prevailing mastitis problems (defined as being among the half of the dairy herds enrolled in the SOMRS with the lowest proportion of cows in udder health classes 0 to 2, corresponding to a cow composite SCC below 200 000 cells/ml during the preceding year), and confirmed cases of *Staph. aureus* and *Streptococcus* spp. mastitis.

Paper III included only herds that sampled at least 75% of even-numbered cows during the sampling period and only cows that were sampled both at the day of calving (D0) and four days later (D4). This resulted in 13 included herds. In paper IV, a slightly larger study population was used as cows from herds that sampled less than 75% of eligible cows were also included. However, all cows had to have been sampled at both D0 and D4 in paper IV as

well, and only cows that were bacteriologically negative (as defined in papers III and IV), or positive for *Staph. aureus*, *Strep. dysgalactiae*, *Strep. uberis*, or the combination of *Staph. aureus*/*Strep. dysgalactiae*, were followed in this paper.

In March and April 2013 four of the 13 included herds in paper III were visited one additional time. The aim was to identify sources of *Staph. aureus* and *Strep. dysgalactiae* on body sites and in the close environment of dry cows and heifers in late pregnancy. Therefore herds included in paper III with a high prevalence of *Staph. aureus* and *Strep. dysgalactiae* were selected.

For papers I and II, all included milk isolates were genotyped and in paper III, a selection of milk isolates from each herd was genotyped. All isolates found in samples from milk, body sites and the environment from the additional visits were genotyped.

3.2 Sampling methods

3.2.1 Milk samples

Isolates used for papers I and II had been stored frozen and were thawed for the current project. Quarter milk samples for papers III and IV were collected by farmers or herd personnel after instructions in aseptic milk sampling technique. These samples were collected before milking at D0 and D4.

At the additional visits, aseptic QMS were collected from all cows that at the time of the visit were in their first or last month of lactation.

3.2.2 Body site and environmental samples at the additional visit

At four farms, samples were collected from body sites of all dry cows, body sites of all heifers within two months of calving, and from the environments of both animal groups. Four body sites (hock skin, teat skin, vagina, and skin wounds) from each animal were sampled with sterile cotton swabs (Amie's charcoal culture swabs; Copan Diagnostics Inc., Murrieta, CA). Hock skin samples were taken by rolling the swab back and forth over the skin of the lateral surface of the right or left hock. If a hock skin wound was present, the sample was taken from the damaged area. Teat skin samples were taken from the same side as the hock sample by rolling a cotton swab down the cranial and up the caudal side of the front teat, and then rolling the same cotton swab in a similar way over the hind teat of the same side. Vaginal samples were taken by inserting a swab approximately 5 cm into the vagina, taking care to avoid contaminating the swab at insertion, and rolling the swab against the mucosal lining. Skin wound samples were taken by gently rolling a swab over the damaged area.

Environmental samples from cubicle walls or stanchion bars, and from feed trough surfaces, were taken by rolling swabs over the area (one cotton swab for every two animals in a pen, up to five samples per pen for cubicle walls and feed troughs, respectively). For the feed troughs, the swabs were rolled over the bottom surface of the trough after removing any feed present. Water cups or troughs were sampled by rolling the cotton swab over the surface of the cup or trough just above the water level (one sample per pen or one sample for every two animals if housed in tie stalls). Bedding material samples were collected manually as follows: three to four handfuls from smaller pens, three composite samples of three to four handfuls from larger pens, and one handful for each animal up to four handful composites from animals housed in tie stalls. Each sample of bedding material was placed into a clean plastic bag.

Disposable gloves were used during all sampling. The gloves were changed between each animal and between each cubicle or group of animals, as well as if the gloves became visibly dirty.

Cotton swabs were moistened with 0.7% NaCl prior to sampling of dry areas (skin without wounds and environmental samples except water cups or troughs). The swabs were placed in Amies medium immediately after sampling. Samples were stored in cooler bags and arrived at the laboratory within 36 hours of sampling.

3.3 Laboratory methods

3.3.1 Sample handling

For all milk samples and previously frozen bacterial isolates, 5% bovine blood agar supplemented with 0.05% esculine (National Veterinary Institute, Uppsala, Sweden) was used as culture medium. For identification of *Staph. aureus* and *Strep. dysgalactiae* in body site and environmental samples, the cotton swab samples were cultured on 5% bovine blood agar supplemented with 0.05% esculine, on mannitol salt agar, and on colistin oxolinic blood agar (COBA).

Samples of bedding material were kept frozen at -20°C. Upon thawing, 5 grams of material was added to 50 ml Nutrient Broth with 10% horse serum and mixed in a stomacher for 2 minutes before being placed in 37°C for four hours. After four hours 10 µl of the broth was spread on 5% bovine blood agar supplemented with 0.05% esculine, mannitol salt agar, and COBA agar. In addition, each sample was diluted tenfold and 10 microliters of the dilution was spread on the same set of agar plates.

After culturing, all plates were incubated overnight at 37°C.

3.3.2 Bacteriological analyses

In papers I and II, bacteriological analyses according to accredited methods were performed previous to the work of this thesis.

In the other studies, isolates were identified according to routine diagnostics by colony morphology and haemolysis. Colonies were considered *Staph. aureus* if morphology was typical and zones of incomplete and complete haemolysis were present. If colony morphology was typical but haemolysis was not, isolates were subjected to a tube coagulase test. Isolates with morphology consistent with that of *Strep. dysgalactiae* or *Strep. uberis* were typed using a set of biochemical reactions and CAMP-reaction. Lancefield grouping and growth on SlaBa (Slanetz & Bartley medium) were used if the results of the biochemical reactions were inconclusive for *Strep. dysgalactiae* and *Strep. uberis*, respectively.

In samples from the additional herd visits, staphylococci not identified as *Staph. aureus* by morphology and haemolysis and all streptococci were identified to species level by Matrix Assisted Laser Desorption Ionization-Time of Flight mass spectrometry (Maldi-Tof). This method was introduced as routine at the National Veterinary Institute, Uppsala, Sweden, in February 2013 for the species identification of udder pathogens.

3.3.3 Genotyping

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis was performed on *Staph. aureus* isolates in paper I, and on *Strep. dysgalactiae* and *Strep. uberis* isolates in papers II and III. In addition, PFGE was performed on *Strep. dysgalactiae* isolates derived from body sites, environmental sites, and milk samples collected at the additional visits.

Macro-restriction patterns derived from PFGE were analysed using computer software. Isolates were considered to be of the same cluster if the similarity level was above 80% (paper II), or if a maximum of three bands differed (paper I and III), and of the same pulsotype when banding patterns were identical. Clusters and pulsotypes received identities depending on species and on in which paper they were included.

spa typing

Single locus DNA-sequencing of the repeat region of the *Staphylococcus* protein A gene (*spa*) was used for genotyping *Staph. aureus* milk isolates in paper III, as well as isolates from body site and environmental samples collected at the additional visits. *spa* typing was performed using primers

spa1113f and spa1514r (Mellmann *et al.*, 2006) or primers spa239f and spa1717r (Hallin *et al.* 2009). *spa* types were assigned using BioNumerics software (BioNumerics Version 7.1; Applied Maths, Inc. 2014; Austin, TX, USA).

3.4 Data editing and statistics

3.4.1 Data editing

In all papers for which an IMI outcome was calculated, mono-infected cows (i.e. cows positive for only one pathogen) were included. In calculations of prevalence and descriptions of occurrence, cows positive for more than one pathogen were also included. In paper III, the latter cows were categorized as co-infected.

In papers III and IV, cows were given a bacteriological status (referred to as bacterial findings) and an infection type on the basis of the results from milk samplings. If any QMS was positive for *Staph. aureus*, *Strep. dysgalactiae*, or *Strep. uberis*, the cow's bacteriological status was positive for that specific pathogen. In paper IV, the combined bacteriological status of *Staph. aureus* and *Strep. dysgalactiae* was added. Cows positive for *Staph. aureus*, *Strep. dysgalactiae*, or *Strep. uberis* were, for each pathogen, allocated to one of the following infection types: cleared (CLE) if the pathogen was present only at D0; persistent (PER) if the pathogen was present at D0 and in the same quarter at D4; new (NEW) if the pathogen was present only at D4, or CLE/NEW if the pathogen was identified in one udder quarter at D0 and another udder quarter at D4.

3.4.2 Statistical methods

Records of parity, breed, culling date, culling reason, test-day SCC, and milk yield at the monthly milk recording for the follow-up period were retrieved from the SOMRS. Records of VTCM were retrieved from the SADR; these records included date of VTCM but no information about udder pathogen. In papers I and II, a 120-day follow-up period was used. In papers III and IV, the first month of lactation and the 305-day lactation were used, respectively.

Associations between CM or IMI and outcome parameters were investigated using the following outcome measurements: SCC (papers I-IV), milk yield (paper I, II, IV), VTCM (papers III and IV), culling (paper IV), and the combined variable of additional VTCMs and culling (papers I and II).

Associations between pathogens (II and IV), genotypes (I and II), and/or infection types (III and IV) and SCC were calculated using multivariable mixed-effects linear regression models, controlling for repeated measurements

of SCC within cow (papers I and II), repeated measurements within herd (paper III), or repeated measurements within herd and cow (paper IV). Test day SCC was transformed using the Box-Cox power transformation. Possible effects of breed, parity, DIM at test milking, and milk yield were considered in the models. In addition, β -lactamase production of *Staph. aureus* was considered in paper I, and the DIM at VTCM in papers I and II.

Linear multivariable mixed-effect regression models were also used in papers I, II and IV to investigate associations between pathogen, genotype, and/or infection type and milk yield. In the milk yield models, test-day SCC was added as an explanatory variable.

Multivariable mixed-effect logistic regression models with repeated measurements of herd were used to evaluate any associations between infection type and VTCM (papers III and IV), and infection type and culling (paper IV). Multivariable mixed-effect logistic regression models were also used to investigate associations between bacterial status for the three pathogens and parity and season.

In papers I, II and III Fisher's exact test and descriptive statistics were also used.

Explanatory variables of primary interest used in the regression models in each paper are presented in Table 1.

Table 1. *Explanatory variables of primary interest used in the regression models of each paper*

Paper	Level	Species	Explanatory variable
I	genotype	<i>Staph. aureus</i>	Common pulsotypes*
I	genotype	<i>Staph. aureus</i>	Common pulsotypes*, less common pulsotypes, rare pulsotypes
I	genotype	<i>Staph. aureus</i>	Common pulsotypes, less common/rare pulsotypes
I	genotype	<i>Staph. aureus</i>	Common clusters*
I	genotype	<i>Staph. aureus</i>	Common clusters*, less common clusters, rare clusters
I	genotype	<i>Staph. aureus</i>	Common clusters, less common/rare clusters
II	species		<i>Strep. dysgalactiae</i> , <i>Strep. uberis</i>
II	genotype	<i>Strep. dysgalactiae</i>	Common clusters*
II	genotype	<i>Strep. dysgalactiae</i>	Common clusters, less common/rare clusters
II	genotype	<i>Strep. uberis</i>	Clusters, non-cluster pulsotypes
III	infection type	<i>Staph. aureus</i>	NEG, CLE, PER, NEW, CLE/NEW ¹
III	infection type	<i>Strep. dysgalactiae</i>	NEG, CLE, PER, NEW, CLE/NEW
III	infection type	<i>Strep. uberis</i>	NEG, CLE, PER, NEW, CLE/NEW
IV	species		NEG, <i>Staph. aureus</i> , <i>Strep. dysgalactiae</i> , <i>Strep. uberis</i> , <i>Staph. aureus/Strep. dysgalactiae</i>
IV	infection type	<i>Staph. aureus</i>	NEG, CLE, NEW, PER
IV	infection type	<i>Strep. dysgalactiae</i>	NEG, CLE, NEW, PER
IV	infection type	<i>Strep. uberis</i>	NEG, CLE, NEW, PER

* Common pulsotypes or clusters were included individually, not as a group

¹See abbreviations list

4 Results

A summary of the results presented in papers I to IV is given below, as well as a detailed description of the results of the additional study. For a more detailed description of the other studies, the reader is referred to each paper.

4.1 National genotype variation in isolates from VTCM

4.1.1 *Staphylococcus aureus* (I)

Among 185 isolates of *Staph. aureus*, PFGE identified 29 pulsotypes. Two pulsotypes were common, accounting for 64% of the material, each pulsotype contributing with 82 and 54 isolates. The remaining pulsotypes were represented by one to two isolates each (called rare pulsotypes; n = 20) or three to 17 isolates each (called less common pulsotypes; n = 7).

Pulsotypes could be grouped into 18 clusters. Of the isolates, 74% belonged to two common clusters.

4.1.2 *Streptococcus dysgalactiae* (II)

Among 132 isolates of *Strep. dysgalactiae*, PFGE identified 71 pulsotypes. Nineteen of the pulsotypes could be found in two to 13 herds each. The remaining 52 pulsotypes were only found once each. Sixty-eight of the pulsotypes could be compiled into nine clusters while the remaining three pulsotypes could not be clustered. Three of the clusters were considered common, represented by 30 to 40 isolates each, and together accounting for 82% of the isolates. The remaining clusters were less common or rare, each represented by two to six isolates.

4.1.3 *Streptococcus uberis* (II)

All 97 isolates of *Strep. uberis* were of different pulsotypes. Forty-five of the isolates belonged to 21 clusters, each found in a maximum of three herds. The remaining pulsotypes could not be clustered.

4.2 Occurrence of udder pathogens in early lactation (III)

4.2.1 Occurrence of bacterial species and infection types

Approximately 26% of primiparous and 31% of multiparous cows had at least one udder quarter infected with *Staph. aureus*, *Strep. dysgalactiae*, and/or *Strep. uberis* at D0 (CLE and PER infections). Herd prevalence ranged from 0 to 42% in primiparous cows, and 11 to 42% in multiparous cows. At D4 (PER and NEW infections), the corresponding percentages were 23 and 29% for primiparous and multiparous cows, respectively, with a herd prevalence range of 0 to 44% in primiparous cows and 13 to 50% in multiparous cows. The most commonly occurring pathogen was *Staph. aureus*, followed by *Strep. dysgalactiae* and *Strep. uberis*. About 25% of cows positive for *Staph. aureus*, *Strep. dysgalactiae*, and/or *Strep. uberis* were co-infected. Among co-infections, the combination of *Staph. aureus* and *Strep. dysgalactiae* was most common.

Persistent infections were most common among *Staph. aureus* positive cows, whereas CLE infections were most common among *Strep. dysgalactiae* and *Strep. uberis* positive cows. Persistent *Staph. aureus* infections were more common in multiparous cows than in primiparous ones. Among *Strep. dysgalactiae* and *Strep. uberis* positive cows, the proportions of PER infections were about equal in both primiparous and multiparous cows.

No overall seasonal patterns were identifiable for *Staph. aureus* infections. *Streptococcus dysgalactiae* infections were least common in September to December, and *Strep. uberis* infections tended to be less common in May to August compared to the rest of the year.

There was a marked variation between herds in the occurrence of pathogens and infection types, which also varied between seasons and parities. However, in all herds *Strep. uberis* was more common among multiparous cows than in primiparous cows.

4.2.2 Genetic variation in isolates collected in early lactation

Genotype patterns varied somewhat with respect to bacterial species and herds. In many herds, two or three *Staph. aureus spa* types were identified, but one *spa* type was often predominating. However, a few herds had a different pattern. In one of them, all isolates were of the same *spa* type, and in two herds seven and four *spa* types were identified. A predominating *Strep. dysgalactiae* pulsotype was only identified in one herd, while none of the herds had a predominating *Strep. uberis* pulsotype. Among *Strep. uberis* isolates, the same pulsotype was rarely found in more than one or two cows, although in two herds, the same pulsotype was identified in four and three cows each.

The same *Staph. aureus spa* type or *Strep. dysgalactiae* or *Strep. uberis* pulsotype was identified in both samples (D0 and D4) from most PER cows. No distinctions in genotype patterns could be identified between infection types for any of the species.

In most herds, the same genotype could be found in cows from different parities when more than a few isolates were genotyped. In three herds, however, all isolates from multiparous cows were of the same *Staph. aureus spa* type (t529), while several *spa* types, including t529, were found in primiparous cows.

Between-herd genotype variation was only studied for *Staph. aureus*. Thirteen *spa* types were identified among 160 isolates from 13 herds. The most common *spa* type was t529, which was found in eleven of the herds.

4.3 Effects of IMI on SCC and/or milk yield

4.3.1 Outcome after clinical mastitis (I and II)

Geometric mean of test-day SCC and mean test-day milk yield during the follow-up period was 150 100 cells/ml and 28.3 kg, respectively, for *Staph. aureus*-cows, 87 900 and 27.5 kg, respectively, for *Strep. dysgalactiae*-cows, and 135 100 and 28.2 kg, respectively, for *Strep. uberis*-cows.

Cows treated for CM caused by common *Staph. aureus* PFGE clusters had significantly lower SCC but tended to have more recordings of additional VTCMs and culling during the follow-up period, compared to cows treated for VTCM caused by less common and rare clusters. No differences in outcome of VTCM could be detected at the *Staph. aureus* pulsotype level.

During the follow-up period, cows treated for VTCM caused by *Strep. dysgalactiae* had significantly lower SCC than cows treated for *Strep. uberis* VTCM. No other differences in outcome could be identified between streptococcal species, genotypes, or genotype groups.

4.3.2 Outcome of IMI (III and IV)

Importance of infection type at calving for udder health in early lactation

Staphylococcus aureus infection types NEW and PER were associated with an increase in test-day SCC during the first month of lactation (Table 2). The same was true for all infection types of *Strep. dysgalactiae* and *Strep. uberis*.

The proportion of cows treated for CM within one month after calving was higher in *Staph. aureus* PER and *Strep. uberis* NEW and PER cows, compared to NEG cows (Table 2).

Table 2. Results of papers III (1st month of lactation) and IV (complete lactation), where ↑ signifies a significant ($P < 0.05$) increase, ↓ a significant decrease, and 0 signifies that no association was identified. Arrows within parenthesis signifies results with P -values $0.1 > P > 0.05$

Bacterial species ¹ , Infection types ²	SCC		VTCM		Milk yield	Culling due to any reason	Culling due to mastitis
	III	IV	III	IV	IV	IV	IV
Sa	- ³	↑	0	0	0	0	0
Srd	-	↑	0	0	0	0	↑
Sru	-	↑	↑	(↑)	0	0	↑
Sa/Srd	-	↑	-	(↑)	0	0	↑
Sa CLE	0	↑*	0	0	0	0	0
Sa NEW	↑	↑*	0	0	0	0	0
Sa PER	↑	↑*	↑	0	0	↑	(↑)
Srd CLE	↑	↑*	0	0	↓**	0	(↑)
Srd NEW	↑	0	0	0	0	0	0
Srd PER	↑	↑*	0	0	(↓)**	↑	↑
Sru CLE	↑	↑	(↑)	0	0	0	0
Sru NEW	↑	↑	↑	0	↑↓***	0	(↑)
Sru PER	↑	(↑)	↑	↑	↓***	0	↑

¹Sa = *Staphylococcus aureus*, Srd = *Streptococcus dysgalactiae*, Sru = *Streptococcus uberis*

²Infection types were defined based upon diagnosis of IMI at day 0 (D0) and day 4 (D4) after calving; infection with a pathogen only at D0 was defined as cleared (CLE), infection only at D4 was defined as new (NEW), and infection with the same pathogen at both D0 and D4 was defined as persistent (PER).

³ - = the explanatory variable was not included in statistical analyses

* Interaction with milk yield **interaction with breed ***interactions with SCC and with parity

Associations between pathogens and infection type at calving, and udder health parameters in subsequent lactation

The geometric mean of SCC during the follow-up period was 70 000 cells/ml for NEG cows, and 131 000, 125 000, 133 000, and 205 000, for *Staph. aureus*, *Strep. dysgalactiae*, *Strep. uberis*, and *Staph. aureus/Strep. dysgalactiae* cows, respectively.

The SCC was significantly higher in cows positive for *Staph. aureus*, *Strep. dysgalactiae*, *Strep. uberis*, and *Staph. aureus/Strep. dysgalactiae* compared to NEG cows (Table 2). Moreover, all *Staph. aureus* and *Strep. dysgalactiae* infection types, and CLE and NEW *Strep. uberis* infection types, had significantly higher SCC than the NEG cows. Interactions between milk yield and bacterial findings and infection types except *Strep. uberis* infection types were detected. The interactions implied that the decrease of SCC with

increasing milk yield was most obvious in NEG cows and significantly less so in certain infection types and pathogens.

Among the NEG cows, 10% were treated for CM during the follow-up period. Corresponding numbers for *Staph. aureus*, *Strep. dysgalactiae*, *Strep. uberis*, and *Staph. aureus/Strep. dysgalactiae* positive cows were 14, 13, 27, and 19%, respectively. The difference between NEG cows and *Strep. uberis* positive cows was significant. Among infection types, VTCM was significantly more common in *Strep. uberis* PER than NEG cows (Table 2).

Associations between pathogens and infection types at calving, and milk yield and culling in the subsequent lactation

Average test-day milk yield was 30.4 kg for NEG cows, and 29.5, 28.9, 30.6 and 30.3 kg for *Staph. aureus*, *Strep. dysgalactiae*, *Strep. uberis*, and *Staph. aureus/Strep. dysgalactiae* positive cows, respectively. There was no overall association between milk yield and bacterial findings or for any of the *Staph. aureus* infection types; however, there was a significant association between milk yield and *Strep. dysgalactiae* and between milk yield and *Strep. uberis* (Table 2). In the model with *Strep. dysgalactiae*, there was an interaction between infection type and breed, implying differences in the effect of infection type on milk yield depending on cow breed.

In the *Strep. uberis* model, infection type interacted with both SCC and parity; the interaction with parity implied that the effect on SCC differed between parities depending on infection type. The interaction between infection type and SCC in this model showed that the milk yield decreased with increasing SCC but that the amount differed between *Strep. uberis* infection types.

During the study period, 162 of 471 cows (34%) were culled. Among NEG cows and *Staph. aureus*, *Strep. dysgalactiae*, *Strep. uberis*, and *Staph. aureus/Strep. dysgalactiae* positive cows, 28, 35, 39, 41, and 43% were culled, respectively. There were no associations between positive cows at bacterial species level and culling for any reason. However, among *Staph. aureus* and *Strep. dysgalactiae* infection types, more PER infected cows than NEG cows were culled for any reason (Table 2).

When using culling due to mastitis as the outcome variable, more *Strep. dysgalactiae*, *Strep. uberis*, and *Staph. aureus/Strep. dysgalactiae*, but not *Staph. aureus*, positive cows were culled (Table 2). Among infection types, more *Strep. dysgalactiae* and *Strep. uberis* PER infected cows than NEG cows were culled due to mastitis, and similar tendencies were identified for *Staph. aureus* PER cows, *Strep. dysgalactiae* CLE cows, and *Strep. uberis* NEW cows.

4.4 Occurrence of udder pathogens in milk, body sites and animal environment at the additional visit

An extended investigation of *Staph. aureus* and *Strep. dysgalactiae* occurrence in milk, on body sites, and in the close environment of the udder and cow in the period around calving was performed in four herds. The results of this extended investigation have not yet been compiled into a scientific paper but are presented here.

4.4.1 *Staphylococcus aureus*

Staphylococcus aureus was found on body sites and/or in environmental samples in all four herds (Table 3), and in milk from cows in early or late lactation in three herds at this additional visit. The most common body site for *Staph. aureus* positive samples was hock skin. This was true for both heifers and dry cows, although the proportion of positive samples for each animal group varied among herds (Table 3).

Findings of *Staph. aureus* in the environment around heifers varied between herds, but most of the samples were negative except those from feed troughs in one herd and samples from cubicle walls/stanchion bars in another (Table 2). In the dry cow environment, it was most common to find *Staph. aureus* on cubicle walls/stanchion bars and in bedding material, and less common in feed and water troughs (Table 3). *Staphylococcus aureus* was also found in bedding material in the calving premises in two of the herds (Table 3).

Sixty-five isolates from body sites and environment and five milk isolates from the additional visit were *spa* typed (Table 4). The most common *spa* types in body site and environmental samples was t529, and this was the only *spa* type identified among the five milk isolates.

4.4.2 *Streptococcus dysgalactiae*

Streptococcus dysgalactiae was found in milk from cows in early or late lactation in two (D and J) of the four herds. In one herd (J), a single QMS was positive for *Strep. dysgalactiae*, while 6 QMS were positive for *Strep. dysgalactiae* in the other (D). In the latter herd, *Streptococcus dysgalactiae* was also found on teat skin in one cow and in a wound from another cow. In both samples a co-infection with *Staph. aureus* was found. All other body site and environmental samples were negative for *Strep. dysgalactiae*.

All *Strep. dysgalactiae* isolates from herd D were genotyped using PFGE. One of the milk isolates and the two body site isolates were of the predominant pulsotype identified in paper III. The remaining milk isolates were of the same cluster, but of another pulsotype, as the predominant pulsotype in the herd.

Table 3. Number of samples with growth of *Staphylococcus aureus*/ total number of samples taken with cotton swabs on body sites and environmental sites, and by collection of bedding material, in 4 herds (C, D, G, and J), divided by animal group and sample site

Animal group	Sample site	Herd				Total
		C	D	G	J	
Heifers ¹	Hock skin	4/6	0/4	6/9	0/3	10/22
	Teat skin	0/6	0/4	1/9	0/3	1/22
	Vagina	0/6	1/4	0/9	0/3	1/22
	Wound	0/4	-	0/1	-	0/5
	Cubicles	0/6	0/5	0/7	4/10	4/28
	Feed troughs	3/6	0/5	0/5	0/10	3/26
	Water troughs	0/4	0/1	0/2	0/2	0/9
	Bedding material	1/1	0/3	0/3	1/6	2/13
Dry cows	Hock skin	4/7	2/10	7/11	1/8	14/36
	Teat skin	2/7	2/10	1/11	0/8	5/36
	Vagina	0/7	0/10	0/11	0/8	0/36
	Wound	-	1/2	0/6	0/5	1/13
	Cubicles	3/10	1/10	5/10 ²	1/5	10/35
	Feed troughs	0/9	2/10	2/9 ³	0/5	4/33
	Water troughs	0/2	0/2	1/3	0/2	1/9
	Bedding material	1/6	3/5	1/5	0/3	5/19
Cows in calving pen	Hock skin	0/1	-	0/2	0/1	0/4
	Teat skin	0/1	-	1/2	0/1	1/4
	Vagina	0/1	-	0/2	0/1	0/4
	Wound	0/1	-	0/2	-	0/3
	Cubicles	0/2	0/5	0/4	0/1	0/12
	Feed troughs	0/2	0/5	0/4	0/1	0/12
	Water troughs	0/2	0/4	0/4	0/1	0/11
	Bedding material	0/1	1/4	2/4	0/1	3/10

¹Heifers within 2 months of expected calving

²Dry cows with previous *Staph. aureus* infections were kept in a group of milking cows with mastitis problems in this herd. All *Staph. aureus* positive samples from dry cow cubicles were from this group in this herd

³Both positive samples were from the above-mentioned group

Table 4. *Staphylococcus aureus* spa types in samples taken with cotton swabs on body and environmental sites, and by collection of bedding material, in 4 herds (C, D, G, and J); animal group and sample site are specified. The number of samples positive for each spa type within each herd and category is given within parentheses.

Animal group	Sample site	Herd			
		C	D	G	J
Heifers	Body	t1403 (2) t529 (2)	t267 (1)	t529 (7)	t267 (1)
	Environment	t1403 (1) t359 (1) t529 (2)	-	-	t267 (1) t13815 (1) untypeable (1) ¹
Dry cows	Body	t529 (6)	t529 (5)	t529 (9)	t267 (1)
	Environment	t529 (4)	t127 (1) t13814 (1) t529 (4)	t529 (9)	t267 (1)
Cows in calving pen	Body	-	-	t529 (1)	
	Environment	-	t529 (1)	t529 (2)	

¹Isolate confirmed as *Staphylococcus aureus* by Maldi-Tof, but was untypeable with two different sets of primers

5 General Discussion

Below follows a discussion of the most important findings in this thesis, with a special focus on the comparative aspects of the different papers. The results from the additional herd visits are also discussed in this section. Please see papers I-IV for detailed reflections of the results of each paper.

5.1 Genotype variation and spread of udder pathogens (I-III)

One aim of this thesis was to investigate the occurrence and genotype variation in Sweden of the udder pathogens *Staph. aureus*, *Strep. dysgalactiae*, and *Strep. uberis*; to increase the knowledge about the infections they cause. This was accomplished by genotyping isolates from cases of VTCM, and by investigating the occurrence of IMI, including genotype variation, at and just after calving in herds with mastitis problems. The occurrence and genotype variation of *Staph. aureus* and *Strep. dysgalactiae* in body sites and environment in a few of those herds was also investigated. A few sample herds (mainly herds D, G, and L) with specific infection patterns are used in the discussion to exemplify within-herd occurrence of pathogens and genotype patterns. To facilitate the comparative discussion of papers I and III, some of the *Staph. aureus* isolates from the national VTCM material were genotyped using both PFGE and *spa* typing. The results of this comparison are unpublished but are used below.

The two most common *Staph. aureus* genotypes in the national VTCM material were found in more than 60% of 185 herds. In contrast, none of almost 100 *Strep. uberis* isolates from different herds were of the same genotype. Among *Strep. dysgalactiae* isolates the variation was intermediate compared to that of *Staph. aureus* and *Strep. uberis*. In IMI at and just after calving in herds with mastitis problems, the overall genotype variation was in line with the results of the national VTCM material; isolates of *Staph. aureus*

showed the lowest genetic diversity, followed by intermediate diversity of *Strep. dysgalactiae* and high diversity of *Strep. uberis*.

5.1.1 *Staphylococcus aureus*

National survey material

Two genotypes of *Staph. aureus* dominated in the national VTCM material, together constituting about two thirds of the studied isolates. Since the isolates were collected in a national survey on the distribution of udder pathogens causing VTCM in Sweden and were epidemiologically independent, the distribution of genotypes presented in this study can be considered representative for Sweden at the time. A limited number of predominating strains associated with most IMI in a region have been identified in several studies previously (Buzzola *et al.*, 2001; Smith *et al.*, 2005; Said *et al.*, 2010). The reason why a few strains become predominant is unknown, but it is speculated that trade of animals can cause a certain genotype to become widespread (Capurro *et al.*, 2010a), possibly in combination with host adaptation and evolvement of specific traits that increase the chance of spread (Smith *et al.*, 2005; Zecconi *et al.*, 2005).

The two most common genotypes (PFGE clusters C11 and C15) in the national VTCM material corresponded well to *spa* types t1403 and t529, respectively, in a comparison between PFGE and *spa* typing (unpublished material). Isolates of the third most common PFGE cluster (C3) were *spa* typed as t267 and t359.

Occurrence of IMI just after calving and of pathogens in extra-mammary samples

In herds with mastitis problems, *Staph. aureus* was the most common udder pathogen. This was expected as it is the most common cause of both CM and SCM in Sweden (Ericsson Unnerstad *et al.*, 2009; Persson Waller *et al.*, 2009; Persson *et al.*, 2011). *Staphylococcus aureus* IMI was common both at the day of calving and as new infections four days later. This was the case in both primiparous and multiparous cows, suggesting that the pathogen spreads among animals prior to first milking as well as among lactating cows.

In herd G, *Staph. aureus* IMI was detected in 44% of the cows at one or both of the two samplings. At the additional visit one year later, the pathogen was also isolated from heifer and dry cow body sites and environment, as well as from milk. All *Staph. aureus* isolates from this herd were identified as *spa* type t529. A similar pattern, with a single *Staph. aureus* genotype causing all IMI in a herd, has been described previously (Sabour *et al.*, 2004; Graber *et al.*,

2009; Capurro *et al.*, 2010b), although a within-herd pattern of a predominant strain co-existing with less common strains seems to be reported more often (Kapur *et al.*, 1995; Sommerhäuser *et al.*, 2003; Tenhagen *et al.*, 2007; Capurro *et al.*, 2010b). Herd G was the only one of the 13 herds where there was a strategy for containment of contagious *Staph. aureus* IMI. Lactating and dry cows that had tested positive for *Staph. aureus* at any time were kept together and segregated from other cows. The lactating *Staph. aureus* cows were milked after non-*Staph. aureus* cows and *Staph. aureus* positive cows could be allowed to stay in the herd for a few years because of these segregation possibilities. The environment of this group was sampled at the additional visit and many of the body site and environment samples were positive for *Staph. aureus*. It has been suggested previously that infection transmission of *Staph. aureus* between cows can occur via flies, fomites, etc. (Matos *et al.*, 1991; Roberson *et al.*, 1994, 1998; Gillespie *et al.*, 1999; Anderson *et al.*, 2012), and it seems possible that such a transmission could occur from a colonized environment as well. Therefore a heavily infested environment such as that of the *Staph. aureus* group in herd G could make up a source of infection also for cows not in the *Staph. aureus* group. Unfortunately, with the current sampling and laboratory protocols, it was impossible to discern if within-herd spread of *Staph. aureus* via for example flies occurred from this environment.

Staphylococcus aureus was also a common finding in both primiparous and multiparous cows in herd D. In this herd, all *Staph. aureus* isolates from multiparous cow IMI and dry cow extra-mammary sites were of one genotype, but multiple genotypes were identified in the IMI and extra-mammary isolates of primiparous cows.

In herd L, *Staphylococcus aureus* was also a common finding, but in this herd most of the IMI isolates were of different genotypes. A pronounced genotype variation of *Staph. aureus* within a herd has been described previously (Sommerhäuser *et al.*, 2003), although more seldom than the more common patterns mentioned above. The pattern of herd L primarily suggests environmental spread of *Staph. aureus*. However, this herd was not visited an additional time and extra-mammary sources were therefore not investigated. Trade of animals with multiple herds could be another possible explanation for the genotype variation in herd L.

Staphylococcus aureus strains isolated from primiparous cows in early lactation have been proposed to be of environmental origin since these animals are not exposed to the risk of contagious spread at milking. However, most of the *spa* types identified in samples from heifer IMI, body sites, and environment were also identified in IMI milk samples of multiparous cows in

the same or other herds. This suggests that strains transmitted to heifer udders from the environment before first milking can be bovine or ruminant specific.

Specific Staphylococcus aureus genotypes found in milk and extra-mammary sites

The most common *spa* type identified in IMI at or just after calving and in extra-mammary sites was t529, followed by *spa* type t267. The third most common *spa* type was t1403, which was only found in IMI in a few primiparous cows in three herds and was only found in three samples from extra-mammary sites, all from heifers in one herd.

It is interesting that the *spa* type corresponding to the most common pulsotype in the national VTCM material (t1403) was much less frequent in the herds with mastitis problems. Clinical manifestation can be associated with bacterial genotype (Fitzgerald *et al.*, 2000; Zadoks *et al.*, 2000; Haveri *et al.*, 2005), and a bias towards another set of genotypes in the problem herds could therefore be possible as isolates from the national material were derived from cases of CM but most isolates from IMI just after calving were associated with SCM. It is also possible that the common *Staph. aureus* genotypes in Sweden differ in their propensity towards spread within herd, resulting in a selection bias for certain genotypes in paper III. In addition, almost ten years had passed between the dates when the two sets of isolates were collected, so a shift over time in genotypes on the national level is possible, as described by Buzzola *et al.* (2001). This should, however, be investigated in a more current nationwide material of CM and SCM.

The use of *spa* and multi-locus sequence typing (MLST) is increasing knowledge about genetic relatedness of *Staph. aureus* strains worldwide. All of the *spa* types mentioned above have been identified in association with mastitis in other parts of the world (Aires-de-Sousa *et al.*, 2007; Said *et al.*, 2010; Mitra *et al.*, 2013; Bar-Gal *et al.*, 2015). *spa* types t267 and t359 belong to one clonal complex (CC; CC97), an old bovine *Staph. aureus* lineage with multiple *spa* types (Hata *et al.*, 2010). *spa* type t529 belongs to CC705, which is a newer complex according to phylogenetic analyses and to the fact that fewer *spa* types belonging to this complex have evolved (Hata *et al.*, 2010). *spa* type t1403 belongs to CC133, a complex that is predominant among Norwegian bovine isolates (Jørgensen *et al.*, 2005). Trade of animals, in combination with adaptation to ruminant environment, are possible reasons for worldwide spread of the more common bovine CCs (Hata *et al.*, 2010).

Two out of four cows with persistent IMI just after calving, from which different *spa* types were identified at D0 and D4, had t267 at one sampling and t359 at the other. t267 and t359 are closely related genotypes; t267 is suggested

to be the clonal ancestor of the other as the result of a point mutation causing a one repeat truncation (Mitra *et al.*, 2013). Evolutionary changes in *Staph. aureus* due to point mutations and deletions may occur during chronic infection in a host (Goerke *et al.*, 2004). This can explain the shifts between genotypes in PER cows, but new IMI with related genotypes are also possible.

Contagious or environmental spread?

Overall, the findings of the thesis suggest that some genotypes of *Staph. aureus* are widespread between countries and herds, and within herds. These genotypes are probably spread from cow to cow at milking, and between herds and countries through trade of animals. However, the same strains also colonize the cow environment, resulting in additional transmission pathways to animals not involved in milking.

In addition, genetic variation of *Staph. aureus* within a herd consistent with environmental pathogens was detected.

5.1.2 Streptococcus dysgalactiae

National survey material

Identical strains of *Strep. dysgalactiae* in different herds has been reported previously (Baseggio *et al.*, 1997; Wang *et al.*, 1999) and in line with that, identical pulsotypes of *Strep. dysgalactiae* were found in multiple herds in our national VTCM material. However, 39% of the isolates belonged to unique pulsotypes. Common environmental sources could explain why the same genotypes were found in multiple herds, but this hypothesis seems less likely for some of the genotypes in this material since identical isolates were found in separate parts of the country. Trade of livestock is extensive within Sweden (Widgren & Frössling, 2010) and perhaps a more likely route of spread of the pathogen between herds. The fly *Hydrotaea irritans*, a vector for *Strep. dysgalactiae* (Chirico *et al.*, 1997), could possibly also be involved in local spread (kilometres).

Occurrence of IMI just after calving and of pathogens in extra-mammary samples

Streptococcus dysgalactiae IMI was commonly found at or just after calving. This was expected as *Strep. dysgalactiae* is a common cause of early lactation CM in both primiparous and multiparous cows in Sweden (Persson Waller *et al.*, 2009). A high occurrence of *Strep. dysgalactiae* soon after calving has also been reported from Norway (Whist *et al.*, 2007).

There were no significant differences in the overall occurrence of *Strep. dysgalactiae* between primiparous cows and multiparous cows. As 11% of primiparous cows were positive for *Strep. dysgalactiae* at the day of calving, the occurrence of prepartum spread of this pathogen seems likely and is in line with previous reports (Aarestrup & Jensen, 1997).

In herd D milk samples, 20% of the cows were positive for *Strep. dysgalactiae* at the day of calving and/or four days later. All genotyped isolates of *Strep. dysgalactiae* IMI at and just after calving in this herd were of the same PFGE cluster. In addition, milk isolates of *Strep. dysgalactiae* collected at the additional visit were of the same PFGE cluster as the cluster identified one year earlier, suggesting stability in the main infectious strain over time. The same *Strep. dysgalactiae* genotype was also found in two dry-cow body sites in this herd, indicating that this genotype also can colonize wounds and teat skin.

In herd G, 33% of the cows were positive for *Strep. dysgalactiae* but the genotype variation of *Strep. dysgalactiae* in this herd was more pronounced than in herd D.

The PFGE results of *Strep. dysgalactiae* isolates collected from IMI just after calving and those collected at the additional visit (isolates from milk and body sites) were compared with PFGE results from the national VTCM material (results not shown). Two of the most common clusters (E and G) from the national material were also found in IMI just after calving. The PFGE pattern of cluster E of the national material was also identified in IMI isolates from herd C and in IMI and body site isolates from herd D. Cluster G of the national material had an identical pattern to the most common genotype in herds A and K. This strongly suggests between-herd spread of *Strep. dysgalactiae*.

Streptococcus dysgalactiae was not found in the environment in any of the additionally visited herds. It is unknown if this was due to the absence of the pathogen in the cows' environment or if the protocol was not suitable for environmental *Strep. dysgalactiae* sampling. This will have to be specifically studied, as no one to my knowledge has yet demonstrated *Strep. dysgalactiae* in the cows' environment.

The between-herd and the within-herd genotype variations of *Strep. dysgalactiae* were similar and indicated some contagious transmission of the pathogen between and within herds. Genotype variation indicating contagious spread has been presented before (Baseggio *et al.*, 1997; Wang *et al.*, 1999) but only in such limited studies that conclusions about the possible spread between herds are not reliable. As mentioned above, a common environmental source could also explain why isolates from different cows or herds have identical

banding patterns. However, this seems unlikely in the current study as one genotype was identified in isolates from 15 different herds and at a ten-year interval.

The genotype variation among IMI at and just after calving in some of the other herds suggested that the pathogen spreads as an environmental pathogen as well. It is unknown if the mode of transmission of *Strep. dysgalactiae* in individual herds is decided by herd-level factors or virulence of specific *Strep. dysgalactiae* strains. Strain differences in virulence factors for *Strep. dysgalactiae*, possibly connected to spread between cows, have been reported (Frost *et al.*, 1977) but this has not been further investigated.

Unfortunately, PFGE of streptococci is not suitable for inter-laboratory comparisons, and therefore comparisons between the current material and strain-typing studies of *Strep. dysgalactiae* from other countries cannot be made. Thus it is unknown if certain *Strep. dysgalactiae* clones are spread worldwide as was described above for *Staph. aureus*.

Contagious or environmental spread?

The results suggest that some genotypes of *Strep. dysgalactiae* are spread within and between herds, and that these strains can be identified in milk as well as on body sites. However, more pronounced genotype variation within and between herds also occurs.

5.1.3 Streptococcus uberis

National survey material

The genotype pattern of *Strep. uberis* isolates from VTCM was heterogeneous in Sweden and we found no evidence of contagious spread between herds as all isolates investigated were of different pulsotypes. Numerous reports state that the genotype variation of *Strep. uberis* is pronounced (Baseggio *et al.*, 1997; Wang *et al.*, 1999; Khan *et al.*, 2003; McDougall *et al.*, 2004; Abureema *et al.*, 2014), therefore the variation in the national survey material was expected. However, a nationwide *Strep. uberis* material had not previously been genotyped.

Occurrence of IMI just after calving

In the current material, *Strep. uberis* was more common in multiparous than primiparous cows. Only a few primiparous cows were *Strep. uberis* positive at the day of calving, suggesting that pre-partum infections with *Strep. uberis* in heifers were not common in these herds. The higher prevalence of *Strep. uberis*

in multiparous cows compared to primiparous cows is in line with the results of previous studies (Zadoks *et al.*, 2001b; Sampimon *et al.*, 2009).

Herd prevalence of *Strep. uberis* varied and herd L was the only herd where *Strep. uberis* IMI was more common than *Staph. aureus* and *Strep. dysgalactiae* IMI. Most of the *Strep. uberis* isolates from this herd were of different genotypes. The overall within-herd genotype variation of *Strep. uberis* in isolates from IMI at and just after calving was less pronounced than in the epidemiologically independent isolates from the national VTCM material. Previous studies identifying occasional isolates with identical banding patterns (Baseggio *et al.*, 1997; Rato *et al.*, 2008) are in line with the within-herd genotype variation of *Strep. uberis* found in this study. It is unknown if this means that there is some contagious spread within a herd, or if the pathogen is transmitted from a common environmental source.

Because the number of *Strep. uberis* positive cows was low in the study-herds, this material was not suitable for a more detailed investigation of within-herd genotype variation or for an international comparison of genotypes.

Contagious or environmental spread?

The findings of the thesis present no evidence for contagious spread of *Strep. uberis* between herds. However, a limited spread between cows within a herd cannot be ruled out as the same genotype was found in more than one cow within a few of the herds.

5.1.4 Herd variations in occurrence of IMI and inter-species comparisons of genotypes

In paper III, infection patterns varied among the 13 herds with mastitis problems in regards to pathogens, infection types, genotypes, parities, and season. Between-herd variations in the overall and pathogen-specific occurrence of IMI and mastitis have been presented previously (Fox *et al.*, 1995; Barkema *et al.*, 1998; Østerås *et al.*, 2006). The reason(s) for variations between herds in the current study are not known. However, the high prevalence of *Staph. aureus* and *Strep. dysgalactiae* with limited genotype variation of both species in herds D and G, and the combination of genetically variable *Staph. aureus* with a high occurrence of *Strep. uberis* in herd K, suggest that there were problems with infectious disease control at milking and hygiene, respectively. Differences in herd management, housing systems, and in overall udder health are explanations reported or discussed in other studies (Myllys & Rautala, 1995; Olde Riekerink *et al.*, 2008; Verbeke *et al.*, 2014).

Herd variations in occurrence of *Staph. aureus*, *Strep. dysgalactiae*, and *Strep. uberis* between samplings and parities also suggested differences in

management and disease control. For example, none of the primiparous cows in herd E was positive for any of the three pathogens at the day of calving, indicating that udder health management for heifers was good in this herd. However, in the same herd around 25% of the primiparous cows and 20% of the multiparous ones had become *Staph. aureus* positive at day four after calving, indicating a quick spread of such infections during early lactation in this herd. Moreover, infections with *Strep. dysgalactiae* and *Strep. uberis* only occurred during the summer months, suggesting that there was good disease control against these pathogens during the remainder of the year in herd E. In contrast, all bacteriologically positive primiparous cows in herd J were positive at both samplings for *Staph. aureus* or *Strep. dysgalactiae*, and all of the positive multiparous cows were positive at the day of calving. This indicates that udder infections established prior to calving in herd J.

The moderate and large genotype variations identified for *Strep. dysgalactiae* and *Strep. uberis*, respectively, differed markedly from that of *Staph. aureus*. Both *Staph. aureus* and *Strep. dysgalactiae* can spread between cows and herds, as discussed above, but the most common *Staph. aureus* genotypes were more widespread between herds than the most common *Strep. dysgalactiae* ones. The reasons are unknown, but the low bacteriological cure rate for *Staph. aureus* compared to *Strep. dysgalactiae* (Pyörälä & Pyörälä, 1998; Sandgren *et al.*, 2008) is a possible explanation as it gives the pathogen more opportunities to spread from one cow to another.

The proportion of *Strep. dysgalactiae* positive cows was lowest in the early housing season (September to December), but the seasonal pattern of IMI varied markedly between herds. The seasonal pattern for *Strep. uberis* also varied among the herds. For the two herds with the largest proportion of positive cows, *Strep. uberis* was most common in the late housing season (January to April) and the overall lowest proportion of *Strep. uberis* infected cows was found during the summer months (May to August), when all Swedish cows must be on pasture according to legislation. The seasonal trend for *Strep. dysgalactiae* IMI, but not *Strep. uberis* IMI, agrees with a Norwegian study on IMI prevalence throughout lactation (Østerås *et al.*, 2006). In that study the highest prevalence of *Strep. uberis* was instead found during summer (June and July). Moreover, in a previous Swedish study on CM, *Strep. uberis* was least prevalent in the late housing season (January to April; Ericsson Unnerstad *et al.* 2009). In a study on CM in Dutch farms, *Strep. uberis* was most common in late summer (August to October), and *Strep. dysgalactiae* in winter/early spring (December to April; Olde Riekerink *et al.*, 2007). Thus, seasonal variations can often be identified, but herd variations are prominent

suggesting that in the design of prevention strategies, a focus on herd-specific variations is more important than general trends.

5.2 Outcomes of VTCM and IMI (I-IV)

Another aim of this thesis was to investigate the outcome of infections with *Staph. aureus*, *Strep. dysgalactiae*, and *Strep. uberis*. This was done using database retrieved outcome measurements following cases of acute VTCM during lactation and IMI around calving.

5.2.1 Outcome as measured by SCC

Somatic cell count was the outcome measurement with most associations with the explanatory variables of primary interest (bacterial genotype, bacterial species, and infection types) throughout the thesis.

National survey material

It was somewhat surprising that the common *Staph. aureus* genotypes rather than less common/rare genotypes were associated with a lower SCC after VTCM in the national survey material. These results are the opposite of Swiss studies demonstrating a genotype B common both within and between herds and associated with a high SCC (Fournier *et al.*, 2008; Graber *et al.*, 2009). However, in Switzerland another genotype (C) is also extensively spread between herds (but not within herds) and shows a lower SCC compared to genotype B. It would be interesting to investigate if the Swiss genotypes B and C correspond to any of the genotypes identified in the current material.

Paper II reports differences in SCC for VTCMs caused by different streptococci. Somatic cell count during the follow-up period was lower in cows veterinary-treated for CM caused by *Strep. dysgalactiae* than *Strep. uberis*. Possible explanations could be a stronger inflammatory response to *Strep. uberis* at the initial infection (Schepers *et al.*, 1997) or a difference in bacteriological cure rates between species (Kalmus *et al.*, 2014). However, the geometric mean SCC after *Strep. uberis* VTCM was well below the commonly used threshold for SCM of 200 000 cells/ml, indicating that most cows treated for CM caused by *Strep. dysgalactiae* and *Strep. uberis* would probably be considered cured using the data of monthly milk recordings.

Intramammary infections just after calving

Intramammary infections at or just after calving were associated with an increased SCC both at the first test-milking within 30 days after calving and during the subsequent lactation. This was true for *Staph. aureus*, *Strep.*

dysgalactiae, and *Strep. uberis*. The combination of *Staph. aureus*/*Strep. dysgalactiae* IMI just after calving was associated with a significantly higher SCC during lactation compared to the other bacterial findings.

A higher SCC for cows with *Staph. aureus*, *Strep. dysgalactiae* IMI, or a combination of the two, than IMI negative cows in early lactation has been reported previously (Whist *et al.*, 2007, 2009; Paradis *et al.*, 2010). A higher lactational SCC for primiparous cows with predominantly *Strep. uberis* IMI in early lactation has also been reported (Compton *et al.*, 2007). However, Pearson *et al.* (2013) found no difference in SCC between monozygous twins with or without *Strep. uberis* IMI close to calving beyond the first month of lactation. Information about outcome after *Strep. uberis* IMI in early lactation multiparous cows has to my knowledge not been reported previously.

For *Strep. uberis* positive cows in the current study, the SCC was significantly increased during the first month of lactation regardless of at which sampling or samplings IMI was found. However, lactational SCC of cows positive at both samplings was not significantly higher than that of negative cows. It is possible that the high percentage of *Strep. uberis* positive cows treated for VTCM resulted in the lack of a significant association between *Strep. uberis* infections detected at both samplings and SCC in the present study, as SCC can be expected to reach a relatively low level after successful treatment of CM (as described in paper III).

5.2.2 Outcome as measured by VTCM

National survey material

In papers I and II, no significant associations between bacterial genotypes or species and the proportion of cows with recorded additional VTCM during the follow-up period were found. The lack of significant association might have been due to the low number of cows in the study material and the fact that few cows had VTCM recorded following the original VTCM. This, in turn, could be due to successful treatments of the original VTCM. It could also be due to a reduced propensity of the farmer to contact a veterinarian for a second VTCM in the same cow, as repeated treatments of mastitis cases is seldom recommended due to low expected cure rate in chronically infected cows (The Swedish Society of Veterinary Medicine (SVS), 2011).

Intramammary infections just after calving

Following IMI just after calving, more cows with *Staph. aureus* and *Strep. uberis* IMI present at both samplings, and *Strep. uberis* IMI present only at day four after calving, compared to negative cows, were veterinary-treated for CM

during the first month of lactation. In the longer follow-up period used in paper IV only cows with *Strep. uberis* IMI present at both samplings had more VTCM registered during the complete lactation compared to negative cows.

The lack of association between IMI at or just after calving and VTCM for *Strep. dysgalactiae* cows was surprising, as *Strep. dysgalactiae* is a common cause of VTCM in Sweden (Ericsson Unnerstad *et al.*, 2009). Further, *Strep. dysgalactiae* was a common finding in the herds of the study and an increase risk of VTCM during lactation associated with *Strep. dysgalactiae* IMI just after calving has been reported (Whist *et al.*, 2007). A possible explanation for the lack of associations between *Strep. dysgalactiae* and VTCM in the current study lies in the herd selection procedure. All the herds had reported cases of CM caused by *Staph. aureus* or *Streptococcus* spp. in the years preceding the study, but there was no information available about which of the *Streptococcus* spp. It is therefore possible that herds with *Strep. uberis* VTCM were overrepresented in the limited number of selected herds. There could also be a difference in the propensity towards progression into CM, SCM or cure, depending on when IMI establishes. In the present study, cows were sampled on the day of calving and four days later, while sampling was performed on day 6 in the study by Whist *et al.* (2007). It is also possible that *Staph. aureus* and *Strep. dysgalactiae* CM were milder than *Strep. uberis* CM and therefore undetected or not veterinary treated in the study herds, but proportions of mild to moderate to severe clinical mastitis have been reported as about equal for the three pathogens (Verbeke *et al.*, 2014). However, *Staph. aureus* bacterial genotype influences clinical manifestation of mastitis (Fitzgerald *et al.*, 2000; Zadoks *et al.*, 2000; Haveri *et al.*, 2005), therefore a predominance of genotypes causing milder mastitis in the studied herds could possibly have introduced a detection bias.

5.2.3 Outcome as measured by milk yield

National survey material

No differences in milk yield at the cow-level were identified for VTCM caused by different genotypes of *Staph. aureus* or *Strep. dysgalactiae*, or between *Strep. dysgalactiae* or *Strep. uberis*. The lack of difference between *Staph. aureus* genotypes in milk yield is curious, as there seems to be a clear difference in virulence as measured by SCC between common and less common genotypes. Reasons for this lack of difference are unclear, but might be caused by things such as differences between genotypes in the number of quarters affected within cow. Differences in the propensity for spread within cow of different genotypes has been presented (Fournier *et al.*, 2008), and the

number of infected udder quarters significantly influence the decrease in cow-level milk yield (Whist *et al.*, 2009).

Intramammary infections just after calving

Associations between IMI just after calving and test-day milk yield were not found for *Staph. aureus*. Associations between *Strep. dysgalactiae* IMI were detected but depended on which sampling or samplings the IMI was found in relation to calving and cow breed. *Strep. uberis* IMI present only four days after calving and at both samplings influenced milk yield, depending on parity and the SCC.

Lack of influence on milk yield in heifers with *Staph. aureus* IMI in early lactation is also reported by Paradis *et al.* (2010) but in contrast, Whist *et al.* (2009) found a negative effect on milk yield associated with *Staph. aureus* early lactation IMI.

In the model investigating association between milk yield and at which sampling or samplings *Strep. uberis* was found in relation to calving, the effect on milk yield differed between parities. Primiparous cows with *Strep. uberis* IMI only four days after calving had significantly higher milk yield than negative primiparous cows, while multiparous cows with *Strep. uberis* IMI detected only at four days after calving or both at calving and four days later had a lower milk yield compared to negative multiparous cows. It is not likely that an IMI four days after calving increases the lactational milk yield for primiparous cows, but it could be that high-yielding primiparous cows were less resistant to short-duration IMI with *Strep. uberis*. Corresponding results are reported for IMI with coagulase-negative staphylococci in early lactation primiparous cows (Piepers *et al.*, 2010). Decreases in milk yield associated with *Strep. uberis* IMI in early lactation have also been reported previously, but only for primiparous cows (Pearson *et al.*, 2013).

5.2.4 Outcome as measured by culling

National survey material

Few cows were culled in the follow-up period of 120 days after VTCM (papers I and II); therefore this outcome was combined with additional VTCMs in those papers. The low number of culled cows could be explained by the relatively short follow-up period. Clinical mastitis is most common in early lactation (Valde *et al.*, 2004; Svensson *et al.*, 2006; McDougall *et al.*, 2007b; Olde Riekerink *et al.*, 2007, 2008; Persson Waller *et al.*, 2009; Verbeke *et al.*, 2014) but many cows are not culled until the end of lactation (Valde *et al.*, 2004). In addition, cows in later lactation that were already pregnant at the

VTCM might have stayed in the herd at least until calving (Rajala-Schultz & Gröhn, 1999; Schneider *et al.*, 2007). Thus, a longer follow-up period for this outcome measurement might have been of value in the calculations.

Intramammary infections just after calving

Intramammary infections with *Staph. aureus*, *Strep. dysgalactiae*, *Strep. uberis*, and the combination of *Staph. aureus*/*Strep. dysgalactiae* at or just after calving were associated with an increase in culling during the lactation. Similar results have been presented (Reksen *et al.*, 2006; Compton *et al.*, 2007; Whist *et al.*, 2007, 2009). However, in the current study, associations were dependent on the type of culling outcome: “culling for any reason” or the outcome “culling due to mastitis”. Moreover, associations were only found for cows positive both at the day of calving and four days later, and not for those positive only once. Cows positive for *Staph. aureus* at both samplings had an increased incidence of “culling for any reason” but not for “culling due to mastitis”. Cows positive for *Strep. dysgalactiae* at both samplings had an increased incidence of both “culling for any reason” and “culling due to mastitis”, while *Strep. uberis* cows positive at both samplings had an increased incidence of “culling due to mastitis”. This was unexpected as in preventive work against *Staph. aureus* culling is often recommended, while this recommendation is not as general for *Strep. dysgalactiae* or *Strep. uberis* positive cows. It is possible that the difference between species and between culling outcomes could originate in differences in subclinical and clinical manifestations, resulting in a greater risk that *Strep. dysgalactiae* and *Strep. uberis* cows are culled due to mastitis in early lactation, compared to *Staph. aureus* cows

5.3 Methodological concerns

Some methodological issues of this thesis have already been addressed, but those that have not are addressed in this section. These are mainly concerned with the selected study populations, the choice of laboratory methods, definitions of positive samples, and the use of outcome measurements.

5.3.1 Herd selection (III and IV)

The aim of paper III was to identify udder infection patterns just after calving in regards to differences between herds, seasons, and parities. To accomplish this, herds with poor udder health had to be identified. We used the criterion that the herd should be among the half of the dairy herds enrolled in the SOMRS with the smallest proportion of cows with low SCC in the year

preceding the study. Thus, the occurrence of IMI identified in this study cannot be interpreted as a general prevalence of early lactation IMI in Sweden. Comparisons with other studies will have to be made with care as it is probable that IMI was more prevalent in these herds than in the average Swedish ones.

In addition, herd-level SCC can be associated with the proportion of specific pathogens causing CM in a herd (Erskine *et al.*, 1988; Barkema *et al.*, 1998; Olde Riekerink *et al.*, 2008). Therefore, when selecting herds on the basis of the proportion of cows with high SCC, it is possible that we selected for one or two of the pathogens over the other(s).

5.3.2 Laboratory methods (I-III)

Bacteriological methods

A number of methods for typing of bacteria to species level exist, and the inter-laboratory variation in choice of methods is extensive. As new methods for typing bacteria to species level have been introduced (Eriksson & Fasth, 2013), the question arises whether results of previous methods are correct. This is especially evident for *Strep. uberis*, as *Enterococcus* spp. and *Lactococcus lactis* have been mistakenly identified as *Strep. uberis* in some laboratories using biochemical methods and selective agars (Domenico *et al.*; Werner *et al.*, 2014). In the studies of this thesis, biochemical methods were used for typing *Strep. uberis* in papers II and III, generating the question of whether some of these isolates were instead *Enterococcus* spp. or *Lactococcus lactis*. It is, however, likely that the extended set of 12 biochemical reactions in addition to CAMP-reaction and SlaBa-plates, used for identification of streptococci result in a higher specificity compared to the often internationally used methods recommended by the National Mastitis Council (NMC, 1999). The ratio of findings of *Strep. uberis* to “other streptococci” (including *Enterococcus* spp. and *Lactococcus* spp.) in routine diagnostics was also similar before and after the introduction of the Maldi-Tof method at the National Veterinary Institute¹. This supports, but does not prove, that the previously used biochemical methods held high specificity, as Maldi-Tof is a method generally more sensitive and specific than traditionally used methods (Raemy *et al.*, 2013; Schabauer *et al.*, 2014).

A few isolates of *Strep. dysgalactiae* and *Strep. uberis* from the national VTCM material in paper II had irregular PFGE patterns or were untypeable and were later tested by Maldi-Tof. The results showed good correlation

1. Charlotta Fasth and Maria Nilsson-Öst, Mastitlab, National Veterinary Institute, Uppsala, Sweden; personal communication

between methods (results not shown), but unfortunately, all isolates from the national VTCM material have not yet been tested by Maldi-Tof.

Genotyping methods

Several genotyping methods for *Staph. aureus* were considered for the work of this thesis. For paper I, PFGE was chosen because 80 of the isolates had already been genotyped using this method (Capurro *et al.*, 2010a). Pulsed-field gel electrophoresis has been used extensively in research and has an excellent typeability, discriminatory power, and easy interpretation (Olive & Bean, 1999; Zadoks *et al.*, 2002; Hallin *et al.*, 2007). However, PFGE is very time-consuming and its use in inter-laboratory comparisons has been questioned (Tenover *et al.*, 1995). The method has been used in a number of larger-scale studies (Buzzola *et al.*, 2001; Mørk *et al.*, 2005; Capurro *et al.*, 2010a) but has its greatest advantage in studies of disease outbreaks (Tenover *et al.*, 1995).

In paper III, we chose *spa* typing for genotyping of *Staph. aureus* isolates instead of PFGE. The reason for this was that *spa* typing is less time consuming (Golding *et al.*, 2008), but has a discriminatory power similar to that of PFGE (Cookson *et al.*, 2007; Hallin *et al.*, 2007; Hata *et al.*, 2010). When *spa* typing a selection of isolates from the national VTCM material used in paper I, we found a good correlation between the methods (results not shown), but poorer correlation has also been presented (Said *et al.*, 2010).

5.3.3 Interpretation of bacteriological findings and infection types (III and IV)

Interpretation of the bacteriological findings was problematic in paper III. Quarter milk samples were collected twice from each cow, resulting in 8 QMS per cow. For only 27 cows did all 8 QMS test negative. In many cows, coagulase-negative staphylococci, *Corynebacterium bovis*, or contamination flora was present in at least one QMS. Cows that were not positive for *Staph. aureus*, *Strep. dysgalactiae*, *Strep. uberis*, or other udder pathogens were therefore categorized based on number of QMS with mixed flora. Mixed-effects linear regression models controlling for herd effect were then used to evaluate if any of the categories including mixed flora could be grouped with completely culture-negative cows. Somatic cell count at first test-milking within 30 days was used as outcome variable and the categorical variable based on number of QMS with mixed flora was the explanatory variable of primary interest. Parity was also included in the model. As there was no significant difference in SCC between culture-negative cows and cows with up to 4 QMS with mixed flora, these cows formed the group negative (NEG) in further statistical analyses of papers III and IV.

Contaminating flora were also a common finding in conjunction with *Staph. aureus*, *Strep. dysgalactiae*, or *Strep. uberis*, either in the same sample or in the other QMS of the cow. In these cases, however, contamination flora was disregarded in the statistical analyses. As all three pathogens can occur on teat skin, cow body parts, milkers' hands and/or in the environment (Calvinho *et al.*, 1998; Zadoks *et al.*, 2002, 2005; Capurro *et al.*, 2010b; Anderson *et al.*, 2012), it is possible that some *Staph. aureus*, *Strep. dysgalactiae*, or *Strep. uberis* in mixed flora were contaminants rather than IMI. Another risk due to contamination is that pathogens causing IMI are outrivalled by the contaminants, resulting in difficulties to detect the pathogens of interest.

Because of the risks of misclassification, two positive samples would possibly be more reliable for diagnosing a true IMI when there is no information about concurrent SCC to differentiate contamination from SCM. However, as pathogens are shed in variable concentrations over time (see review by Britten, 2012), two positive samples would lower the sensitivity. Indeed, definitions of IMI proposed and used in the literature range from growth of a pathogen in pure culture or in mixed flora in a single sample, to the results of triplicate sampling, to define IMI (Erskine & Eberhart, 1988; Dingwell *et al.*, 2003; Hillerton *et al.*, 2007; Dohoo *et al.*, 2011a; b).

Co-infections were also of concern. It can be argued that the occurrence of more than one pathogen in a sample might reflect that one or both of the occurring pathogens are contaminations. Thus, as the importance of co-infections is unknown, co-infection should not be included in any calculations. However, in the present material, co-infections were common: for example, more than half of *Strep. dysgalactiae* isolates occurred as co-infections. If all co-infections were excluded there would have been a risk of missing important infection patterns as it is possible that the species of co-infections are of importance for udder health and/or reflect the infection load of these species within a herd.

Co-infections of a combination of *Staph. aureus*, *Strep. dysgalactiae*, and/or *Strep. uberis* have been described as common (Barkema *et al.*, 1998; Whist *et al.*, 2007; Keane *et al.*, 2013), but the prevalence of co-infections in IMI just after calving was higher than expected in our material. The high occurrence was probably caused by the wide definition of co-infections. Our study included cows that were positive for more than one pathogen on cow-level, and disregarded if the pathogens were present at the same sampling or not. As it is not known if one or both pathogens in a co-infection is of importance for udder health and milk production, co-infected cows were excluded from statistical analyses of such parameters in paper III. In paper IV, only the most common

co-infection (*Staph. aureus/Strep. dysgalactiae*) was included in the analyses of outcome after IMI.

5.3.4 Outcome measurements (I-IV)

Parameters used as outcome measurements in this thesis were chosen to reflect udder health in the absence of follow-up visits with bacteriological sampling and/or clinical examination of the cows. Although these measurements are indirect measurements of cure, all are of importance in practice, because high SCC, decreased milk yield, CM, and culling are all associated with increased costs. The use of these parameters as outcome measurements can, however, be questioned for various reasons.

First, all database parameters are recorded on cow-level. As there is often a decrease in milk yield in the affected udder quarter (Tesfaye *et al.*, 2010; Botaro *et al.*, 2014), or even a non-functional quarter (Waage *et al.*, 2000; Compton *et al.*, 2007), a lower yield of high SCC-milk will be diluted by the milk produced in the healthy quarters. Thus, an udder quarter with an increased SCC might be missed when using cow composite SCC as outcome measurement (Berglund *et al.*, 2004).

Registered cases of VTCM retrieved from SADRIS is a somewhat unspecific outcome measurement, as the database does not include information about which udder quarter is affected or which pathogen was found at bacteriological culturing. Thus, it is unknown if the VTCM registered in the follow-up periods was associated with the original VTCM (papers I and II) or with IMI in early lactation (papers III and IV). The VTCM parameter can also be questioned due to the subjectivity of the measurement, as several factors influence the decision of a farmer to contact a veterinarian or not (Mörk *et al.*, 2009). In addition, if clinical symptoms are subtle, a case might be missed altogether. Another problem with using VTCM registered in the SADRIS as outcome is that only about 78% of cases of VTCM are recorded in the database (Wolff *et al.*, 2012).

Milk yield as outcome can be questioned when pre-infection milk yield or genetic merit of milk yield is not included in the statistical models, as higher yield is associated with an increased risk of mastitis (Gröhn *et al.*, 2004; Hagnestam *et al.*, 2007). Therefore, high-yielding cows might be overrepresented in bacteriologically positive cows compared to negative cows, and a decrease in milk yield following IMI might not be detected. Unfortunately, information about pre-IMI or pre-VTCM milk yield was not possible to include in the current studies, as there is no information about pre-infection milk yield for primiparous cows before first test-milking, and information about genetic merit for milk yield was not available.

Finally, culling decisions may be influenced by factors other than diseases, such as the number of available heifers in the herd, meat prices, and pregnancy status of the cow (Lehenbauer & Oltjen, 1998; Groenendaal *et al.*, 2004; Schneider *et al.*, 2007). In addition, recorded culling reason are subjectively chosen by the farmer, and can therefore vary depending on individual strategies. In mastitis-related studies, “culling for any reason” and “culling due to mastitis” can be used as outcome parameters. As it has been shown that mastitis, especially in early lactation, is associated with other health parameters such as reproduction (Barker *et al.*, 1998; Schrick *et al.*, 2001; Santos *et al.*, 2004), the less specific outcome parameter “culling for any reason” may be justifiable. However, as a culling decision is based on many factors, “culling due to mastitis” might be a better measurement of udder health following IMI or mastitis. In papers I and II, “culling due to mastitis” was the only culling outcome used, because of its higher specificity as an udder health measurement. In paper IV, both outcomes were investigated because of the possible impact of early lactation IMI on reproduction (Barker *et al.*, 1998; Schrick *et al.*, 2001; Santos *et al.*, 2004).

5.4 Practical applications

The overall aim of the thesis was to generate better knowledge for the use in prevention of mastitis caused by *Staph. aureus*, *Strep. dysgalactiae*, and *Strep. uberis*. Below, some practical applications of the generated results are highlighted.

5.4.1 General recommendations

The predominant source of *Staph. aureus* IMI in Sweden seems to be the infected udder. It can also be concluded that *Strep. uberis* IMI predominantly seems to be of environmental origin. Thus, first-hand preventive measures against these pathogens can remain the same as the present recommendations.

The predominant source of *Strep. dysgalactiae* is, however, not as evident as for the two other pathogens and the presented results suggest that *Strep. dysgalactiae* cannot be categorized as predominantly contagious or environmental.

5.4.2 Spread of infections within herds

Infection patterns in early lactation are herd-specific, therefore general recommendations on how to prevent IMI might not be sufficient. As described above, milk sampling for bacteriology of newly calved cows at the day of calving and four days later can be of value to identify infection patterns

indicating when IMI establishes, e.g. before calving or during the first days of lactation. When sampling multiparous cows, sampling at drying-off should probably also be added to the protocol.

Recommendations on milk sampling and bacteriological methods

Diagnosis of IMI can be costly, especially if quarter milk samples are used. An alternative to quarter milk sampling is to collect cow composite samples. This saves costs, but the risk for contamination is greater, and there is a risk of loss of sensitivity due to dilution of the milk (Reyher & Dohoo, 2011).

Culturing is the routine method to investigate bacterial growth in milk samples. During recent years, detection of udder pathogens by polymerase chain reaction (PCR) has been introduced. This method is faster and more suitable for the analysis of composite milk samples as it can detect lower concentrations of bacteria. However, when using culture-independent methods such as PCR for diagnosing IMI on the species level, the possibilities of genotyping and further testing of antimicrobial susceptibility are lost.

The value of genotyping udder pathogens in practice

Genotyping udder pathogens is expensive today, but faster and less expensive methods are being developed and evaluated (Boss *et al.*, 2011).

With available methods, genotyping of *Staph. aureus*, *Strep. dysgalactiae*, and *Strep. uberis* could be of value in herds where general preventive measures are unsuccessful and where bacteriological sampling has not indicated either contagious or environmental spread. Neither *spa* typing nor PFGE is used in routine mastitis diagnostics today, but with increased demand, faster and cheaper methods will become available in the future.

Even if genotyping in most cases is too expensive to apply at the start of an investigation into mastitis problems in a herd, milk samples or bacterial strains could be frozen for further diagnostic tests if warranted.

5.4.3 Identifying cows at risk for udder health problems

In herds with mastitis problems, bacteriological analyses of milk samples taken from cows just after calving can be used as a tool to segregate healthy cows from non-healthy cows, to prevent spread of infections. Such analyses can also be used to identify cows at risk for udder health problems during lactation. As was shown in this thesis, occurrence of *Staph. aureus*, *Strep. dysgalactiae*, and *Strep. uberis* just after calving is associated with an increase in SCC throughout lactation. By identifying risk cows, suitable management decisions can be taken such as whether the animal should be segregated, treated, or inseminated.

Intramammary infections just after calving were associated with an increase in SCC at first test-milking and throughout lactation. Therefore, the results of the first test-milking of lactation could probably be used as an indicator of udder health during the rest of lactation. When bacteriological analyses of milk cannot be afforded, results of the first-test milking could guide decisions regarding, for example, segregation and insemination. However, as the increases in cow level SCC following IMI in early lactation were small (as evidenced by the relatively low geometric mean following IMI with all pathogens except the combination of *Staph. aureus/Strep. dysgalactiae*), a lower threshold than 200 000 cells/ml would probably have to be used. Indeed, Østerås *et al.* (2008) showed that there was an increased risk of finding *Strep. dysgalactiae* in cows with a cow composite SCC of above 50 000 cells/ml.

To investigate udder health in newly calved cows, the California Mastitis Test (CMT) is often used to identify udder quarters with increased SCC. Such udder quarters can then be sampled for bacteriological analysis. Selection of udder quarters by using CMT is used to reduce the costs for these analyses. However, as CMT only gives a rough estimate of the SCC, it is possible that quarters with a slightly or moderately increased SCC due to infection are not detected.

According to Swedish and Nordic recommendations on the use of antimicrobials, treatment of IMI without concurrent clinical signs during lactation is not recommended (NMSM (Nordiske Meieriorganisasjoners Samarbeidsutvalg for Mjølke kvalitetsarbeid), 2009; The Swedish Society of Veterinary Medicine (SVS), 2011), based on treatment costs and low cure rates (Sandgren *et al.*, 2008). In addition, the success rate of pre-calving treatment of heifers is dependent on herd and predominant pathogen, and is not always successful as reviewed by De Vliegher *et al.* (2012). However, the effect of treatment of IMI in the period just after calving specifically, when SCM is associated with great costs due to the negative effect on milk yield throughout lactation (Archer *et al.*, 2013), has not been investigated. This could be an interesting topic for future research, although, as there is increasing concern about the use of antimicrobials in livestock, prevention remains the best way to handle mastitis problems.

6 Conclusions

The results from the studies presented in this thesis give insight into the genotype variation of *Staph. aureus*, *Strep. dysgalactiae*, and *Strep. uberis* in Sweden and into the main transmission routes of these pathogens, as well as into the long-term outcome of these IMI. Important conclusions of the thesis are that:

- Genotype variation of *Staph. aureus*, *Strep. dysgalactiae*, and *Strep. uberis* is pathogen-dependent. The variation and infection patterns of *Staph. aureus* suggest that infected udders are the main reservoirs of infection for this pathogen, and that contagious spread both within and between herds is common. However, environmental sources were possible within some herds. The patterns of *Strep. dysgalactiae* suggested contagious spread between and within some herds, but environmental spread was suggested in other herds. Isolates of *Strep. uberis* showed high diversity, suggesting that the environment is the main source of this pathogen.
- The common genotypes of *Staph. aureus* were associated with a lower SCC after VTCM compared to less common *Staph. aureus* genotypes. *Streptococcus dysgalactiae* VTCM was associated with a lower SCC in the follow-up period compared to *Strep. uberis* VTCM.
- Intramammary infections at or just after calving were common in herds with mastitis problems. Intramammary infections with *Staph. aureus* and *Strep. dysgalactiae*, but not *Strep. uberis*, were common in primiparous cows on the day of calving, suggesting transmission of *Staph. aureus* and *Strep. dysgalactiae* before the start of first lactation.
- Intramammary infections just after calving were associated with increased SCC during the first month of lactation, as well as throughout the lactation, but associations with other outcome were variable, depending on pathogen, on at which sampling or samplings IMI was found in relation to calving, and on breed and parity.

7 Future perspectives

During the work of this thesis some new questions were raised which could be topics of future research:

Do common and less common/rare Staph. aureus genotypes in Sweden differ in the expression of virulence genes?

In the current study, we found that common *Staph. aureus* genotypes were associated with a lower SCC after VTCM compared to less common/rare genotypes. A number of *Staph. aureus* virulence genes have been described that are associated with outcome of disease (Zecconi *et al.*, 2005; Fournier *et al.*, 2008). Thus, it would be interesting to study if the less common genotypes of our material carry another set of virulence genes compared to the common genotypes. A study that includes both virulence gene expression and cattle movement would be valuable to increase knowledge about what makes some genotypes more widespread than others.

Are any of the widespread Swedish Staph. aureus genotypes equivalent to the Swiss Genotypes B or C?

In a number of Swiss studies (Fournier *et al.*, 2008; Graber *et al.*, 2009; Boss *et al.*, 2011), a PCR-based method has revealed two widespread genotypes in Switzerland, B and C, with different clinical and epidemiological characteristics. Genotype B shows a high within-herd and within-cow prevalence and is associated with an increase in SCC compared to genotype C, which is associated with low within-herd and cow prevalence. These genotypes also differ in regards to set of virulence genes. From the studies within the work of this thesis, a corresponding between- and within-herd genotype pattern was not found. In contrast, more than one genotype was often found in multiple cows within a herd and although *spa* type t529 was most prevalent in almost all herds, the genotype occurred at the same proportion as other genotypes in some herds. However, the method developed for identifying genotypes B and C in

bulk tank milk is relatively cheap and fast (Boss *et al.* 2011) and the results can be used to guide farmers in herd-specific handling of mastitis-problems due to *Staph. aureus*. Therefore, it would be interesting to investigate if the method would have any use in Swedish dairy herds.

Is there an ongoing shift of common Staph. aureus genotypes in Sweden?

Shifts in genotype prevalence among *Staph. aureus* isolates within a region have been reported (Buzzola *et al.*, 2001) and when genotyping *Staph. aureus* isolates from 2002/2003 and from 2011/2012 during the performance of this thesis project, the difference in genotype prevalence between the sets of isolates was striking. However, as isolates were selected by completely different inclusion criteria no conclusions could be drawn from this finding. It would therefore be interesting to compare genotype prevalence in the national VTCM material from 2002/2003 with a newer, comparable, material. Preferably, this comparison would be done using a method which generates results that can be used in larger-scale international comparisons as well.

Further studies on Strep. dysgalactiae

Previous studies (Basseggio *et al.*, 1997; Wang *et al.*, 1999) as well as the results of this thesis, suggest that *Strep. dysgalactiae* to some degree can spread in an environmental fashion. However, no one has presented results of environmental occurrence of the pathogen, as has been done for *Strep. uberis* (Zadoks *et al.*, 2005; Lopez-Benavides *et al.*, 2007) and *Staph. aureus* (Matos *et al.*, 1991; Roberson *et al.*, 1994, 1998; Capurro *et al.*, 2010b; Anderson *et al.*, 2012). Thus, while studying the genetic variation and spread of *Strep. dysgalactiae*, the question of whether *Strep. dysgalactiae* can be cultured from the environment was raised. In addition, the questions of if widespread *Strep. dysgalactiae* genotypes from Sweden can be identified in other countries and if there is a difference in virulence factors in widespread strains compared to less widespread strains were raised.

Can Strep. uberis act as a contagious pathogen in Sweden?

In the current studies, we found no indications of contagious spread of *Strep. uberis* between herds, but within herds two or three cows with the same IMI genotypes were sometimes found. As the prevalence of *Strep. uberis* was low in the study herds, conclusions of within-herd spread were drawn with caution. Studies from other parts of the world suggest that main transmission route of *Strep. uberis* differs between regions (Zadoks *et al.*, 2003; McDougall *et al.*, 2004), from a highly heterogeneous genotype pattern in New Zealand to reports of possible contagious spread in Europe. As management systems differ

between Europe (including Sweden) and New Zealand, a pattern more like that described from the Netherlands would have been expected in Sweden. Therefore, a study using Swedish herds with specific *Strep. uberis* problems could contribute to valuable knowledge about if there are other transmission pathways of *Strep. uberis* in Sweden in addition to environmental spread.

Can antimicrobial treatment of IMI in early lactation be of value?

Antimicrobial treatment of IMI without clinical signs during lactation is not recommended due to low expected cure rates and high costs (Sandgren *et al.* 2008), but the effect of treatment of IMI just after calving specifically has not been investigated. In the current studies, IMI in early lactation was shown to be of importance for udder health and production throughout lactation. It is possible that the cost of lactational antimicrobial treatment could be acceptable if treatment cancelled these negative effects of early lactation IMI. In addition, if immediate treatment resulted in bacteriological cure, the risk of spread of infection to other animals would disappear. A future study of the possible benefits of selective treatment based on bacteriological diagnosis of IMI in early lactation as a part of an udder health programs in selected herds would be of interest.

8 Populärvetenskaplig sammanfattning

Bakgrund

Mastit (juverinflammation) är en vanlig sjukdom hos mjölkkor. Den kan visa sig med kliniska symptom (allt från ändrat utseende på mjölken till systemisk sjukdom; klinisk mastit) eller märkas endast genom förändringar i mjölkens sammansättning (subklinisk mastit). Vid subklinisk mastit är den mest framträdande förändringen en förhöjning av mjölkens celltal som främst sker genom ett inflöde av vita blodkroppar till juvret. Förändringarna i mjölkens sammansättning vid mastit ger en försämring av mjölkens kvalitet vilket innebär att mjölken får ett minskat värde som livsmedel. Dessutom minskar mjölkproduktionen vid mastit vilket innebär ett ekonomiskt bortfall för djurägaren.

En rad bakterier kan orsaka mastit, men den relativa betydelsen av olika bakterier varierar mellan länder, regioner och besättningar. I Sverige hör *Staphylococcus (Staph.) aureus*, *Streptococcus (Strep.) dysgalactiae* och *Strep. uberis* till de vanligaste mastitörsakande bakterierna. Den relativa betydelsen av olika bakteriearter kan också variera mellan årstider och yngre och äldre kor, vilket troligen speglar skillnader i riskfaktorer för de olika bakterierna.

Mastitbakterier delas ofta in i smittsamma och miljöbundna bakterier utifrån deras huvudsakliga smittkälla (juvret respektive miljön). Juvrbundna bakterier smittar mellan djur framför allt vid mjölkningen, medan miljöbundna bakterier huvudsakligen når juvret från kons närmiljö mellan mjölkningarna. Indelningen har betydelse för förebyggande av infektioner och *Staph. aureus* och *Strep. dysgalactiae* har i Sverige traditionellt räknats som smittsamma bakterier medan *Strep. uberis* har räknats som en miljöbunden bakterie. *Staphylococcus aureus* hittas dock ofta i ladugårdsmiljön och *Strep. uberis* har även visat sig kunna orsaka smittsamma utbrott inom besättningar.

Med ny molekylärbiologisk teknologi har kunskapen om de tre bakterierna växt. Den genetiska diversiteten inom en bakterieart speglar huvudsaklig

smittspridning. Smittsamma bakterier visar en låg variation medan miljöbundna bakterier visar en stor genetisk variation inom en population. En viss kunskap finns om genotypers spridning för *Staph. aureus* och *Strep. uberis*, men de svenska förhållandena är till stor del okända. Mycket liten kunskap finns generellt om *Strep. dysgalactiae*-genotyper.

Generellt ger mastit förhöjt celltal i mjölken under en varierande lång tid efter genomgången infektion, minskad mjölkproduktion och ökad risk för utslagning (slakt). Behandlingsresultat, liksom spontan utläkningsförmåga, beror dock på en rad bakteriella faktorer och på kofaktorer. På senare år har man visat att utläkning även kan variera med bakteriell genotyp.

Det huvudsakliga syftet med studierna i denna avhandling var att genom ökad kunskap om genetisk variation och smittspridning av *Staph. aureus*, *Strep. dysgalactiae* och *Strep. uberis* göra arbetet med att förebygga mastit effektivare i framtiden.

Studier och resultat

I de första studierna (I och II) undersöktes den genetiska variationen hos tidigare insamlade isolat av *Staph. aureus*, *Strep. dysgalactiae* och *Strep. uberis* från en nationell studie av veterinärbehandlade kliniska mastiter. För att inte besättningsförekomst av mastit skulle påverka resultatet ingick endast ett isolat per besättning och bakterieart. Isolaten från veterinärbehandlade kliniska mastiter användes också för att undersöka genotypers och bakteriearters (streptokockerna) inverkan på behandlingsresultatet. Behandlingsresultatet utvärderades genom att följa celltal och mjölkproduktion under en 120 dagar lång uppföljningsperiod. Antal nya mastiter och utslagningar på grund av juverhälsa studerades också.

Resultaten från dessa studier visade att den genetiska variationen var relativt liten hos *Staph. aureus* och hög hos *Strep. uberis*. Variationen hos *Strep. dysgalactiae* var intermediär i förhållande till *Staph. aureus* och *Strep. uberis*. Kor som behandlats för mastit orsakade av vanliga *Staph. aureus*-genotyper hade lägre celltal under uppföljningsperioden jämfört med kor som behandlats för mastit orsakat av ovanliga genotyper. En liknande skillnad sågs mellan kor som behandlats för mastit orsakad *Strep. dysgalactiae* jämfört med *Strep. uberis*. Inga skillnader sågs mellan streptokockgenotyperna.

I studie III och IV ingick större lösdriftsbesättningar med juverhälsoproblem. Under en tolv månaders period provtogs hälften av djuren två gånger i tidig laktation, dels innan första mjölkningen efter kalvningen, dels fyra dagar senare. Fyra av besättningarna besöktes även för mjölkprovtagning samt provtagning av hud på olika delar av kroppen samt miljö vid ett tillfälle ett år senare. Denna provtagning bidrog till ett extra material som presenteras i

avhandlingen. Ett urval av isolat från mjölkproverna och alla övriga isolat genotypades.

Resultat från studie III visade att det fanns tydliga skillnader i förekomst av *Staph. aureus*, *Strep. dysgalactiae* och *Strep. uberis* mellan gårdarna, och att det fanns vissa skillnader mellan yngre och äldre kor samt mellan årstider. Både *Staph. aureus* och *Strep. dysgalactiae* var vanliga i prover som togs samma dag som kalvning och i proverna som togs fyra dagar senare. *Streptococcus uberis* var generellt mindre vanlig och var vanligare än de andra två bakterierna i endast en besättning. Denna bakterie var ett ovanligt fynd hos förstakalvare, både vid kalvning och fyra dagar senare. Den genetiska diversiteten hos *Staph. aureus* och *Strep. dysgalactiae* varierade mellan besättningar. Det förekom besättningar inom vilka variationen var mycket liten, besättningar där en viss variation fanns men där också olika kor kunde vara infekterade med samma genotyp, och besättningar med stor genetisk variation bland bakterieisolaten. Den genetiska variationen hos *Strep. uberis* var hög inom besättningarna.

Staphylococcus aureus var vanligt förekommande i miljön och var oftast av samma genotyp som de som hittades vid juverinfektioner på samma gård. *Streptococcus dysgalactiae* hittades inte i miljön men däremot i två kroppsprøver. Dessa isolat var av en i mjölken vanligt förekommande genotyp.

Infektioner med *Staph. aureus*, *Strep. dysgalactiae* och *Strep. uberis* i tidig laktation var förknippade med ett förhöjt celltal både under första laktationsmånaden och under resterande laktation, oavsett om en infektion hittades bara vid kalvning, bara på fjärde dagen, eller vid båda provtagningarna. Bakterieförekomsten vid eller precis efter kalvning var också förknippad med ökad risk för kliniska mastiter, nedsatt mjölkproduktion och ökad utslagning, men detta berodde på vilken bakterie som hittades och sågs främst för de kor som hade samma juverinfektion vid båda provtagningarna.

Slutsatser

Sammantaget tyder resultaten på att några *Staph. aureus*-genotyper är väl spridda i Sverige. Dessa sprids troligen främst direkt mellan djur inom besättning och spridning mellan besättningar sker troligen främst via handel med djur. I vissa besättningar är dock den genetiska variationen hos *Staph. aureus* stor. Den genetiska variationen hos *Strep. dysgalactiae* var större än hos *Staph. aureus* men vissa genotyper föreföll spridas från djur till djur inom och mellan besättningar. Variationen hos *Strep. uberis* var uttalad och vi hittade inga tecken på att denna bakterie sprider sig smittsamt i Sverige. En sådan spridning är dock inte utesluten med tanke på att ganska få gårdar och isolat undersöktes. Eftersom gårdsvariationen var stor vad gäller förekomst av olika

bakterier och när dessa först kunde hittas (vid kalvning eller fyra dagar senare), samt vad gäller säsong, äldre och yngre djur, samt bakteriell genotyp, är det troligt att detaljerad kunskap om infektionsmönster i varje enskild besättning är av värde för att kunna ta fram för besättningen mest lönsamma förebyggande åtgärder.

Staphylococcus aureus-genotyp har betydelse för behandlingsresultatet efter klinisk mastit. Detta bör undersökas vidare för att på sikt kunna förutsäga prognosen bättre för behandling av klinisk mastit orsakad av *Staph. aureus*. Förekomst av juverinfektioner vid eller strax efter kalvning orsakade av *Staph. aureus*, *Strep. dysgalactiae* och *Strep. uberis* har betydelse för juverhälsan under resterande laktation.

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