Acta Universitatis Agriculturae Sueciae

SLU

Doctoral Thesis No. 2020:23
Faculty of Veterinary Medicine and Animal Science

Udder cleft dermatitis in dairy cows

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Doctoral thesis
Swedish University of Agricultural Sciences
Uppsala 2020

Acta Universitatis Agriculturae Sueciae
2020:23

Cover: Uppländsk Swedish Red cow on pasture.
(photo: Peder Strandh, *Uppsalamagasinet Luthagsnytt*)

ISSN 1652-6880
ISBN (print version) 978-91-7760-564-5
ISBN (electronic version) 978-91-7760-565-2

Print: SLU Service/Repro, Uppsala 2020

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Abstract

Udder cleft dermatitis (UCD) is a skin condition that affects dairy cows at the fore udder attachment or between the udder halves. The lesions range from mild (eczematous skin changes) to severe (open wounds and large crusts) and may impair the welfare of the affected animals. This thesis aimed to increase the understanding of the epidemiology and etiology of UCD in Swedish dairy cows.

In the first part of the project, we assessed the prevalence of mild and severe UCD and found that the condition is common in Swedish dairy cows, and we also found several cow- and herd-related risk factors associated with having UCD. Some risk factors were common to both mild and severe UCD, whereas some only affected one type of UCD.

In the second part of the project, we investigated the incidence, duration and recovery of UCD in seven herds, and analysed cow-related factors associated with transitions from being unaffected to having UCD, as well as factors associated with recovery. The incidence was high in the investigated herds, and risk factors for a transition to UCD were similar to those associated with having UCD. The duration of UCD was often long, particularly if the cow had severe UCD. Cows with severe UCD and cases with long duration had low chance of spontaneous recovery from UCD, and older cows also had a lower chance of recovery than younger cows. We also performed a study in four herds, testing a spray with copper and zinc as a topical treatment of UCD, but found no positive effect on UCD recovery in the treated cows compared to the control group.

In addition, using shotgun metagenomic sequencing of swab samples, we investigated the microbiota in UCD lesions in comparison to healthy skin, and found that UCD lesions had a decreased bacterial diversity compared to healthy skin but that no specific pathogen was associated with the development of UCD.

Associations between UCD and mastitis were analysed, and a transition to severe UCD was associated with an increased risk for mastitis, but mastitis-causing pathogens were not a common finding in the UCD microbiota.

In conclusion, this project has led to an increased understanding of the epidemiology and etiology of UCD in Swedish dairy cows that can be used in prevention of the disease.

Keywords: ulcerative mammary dermatitis, necrotic dermatitis, udder sores, cattle, epidemiology, etiology, metagenomic sequencing, microbiota, mastitis

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Juversår hos mjölkkor

Sammanfattning

Juversår är en hudåkomma som ses vid den främre juveranfästningen eller mellan juverhalvorna hos mjölkkor. Såren varierar i utseende, från lindriga (eksemliknande förändringar) till kraftiga (med öppna sår och stora krustor) och de kan medföra försämrad djurvälfärd. Målet med denna avhandling var att öka förståelsen för epidemiologin och etiologin för juversår hos svenska mjölkkor.

I den första delen av projektet undersökte vi förekomsten av lindriga och kraftiga juversår och fann att åkomman är vanlig bland svenska mjölkkor. Vi fann också flera faktorer, både på ko- och besättningsnivå, som hade samband med en ökad risk för att ha juversår. Vissa riskfaktorer hade samband med både lindriga och kraftiga sår, medan andra faktorer bara påverkade en typ av juversår.

I den andra delen av projektet undersökte vi incidens, duration och avläkning av juversår i sju besättningar. Vi undersökte också olika kobundna faktorer och hur de påverkade risken för att gå från att vara frisk till att få ett juversår samt faktorer som påverkade avläkning. Incidensen var hög i de undersökta besättningarna och riskfaktorer för att få juversår liknade de som visats ha samband med att ha juversår. Durationen var ofta lång, speciellt för kraftiga sår. Kor med kraftiga juversår och sår med lång duration hade låg sannolikhet att avläka spontant och dessutom hade äldre kor lägre sannolikhet för avläkning än yngre kor. Vi utförde också en studie i fyra besättningar där vi testade en sårspray innehållande zink och koppar som behandling mot juversår, men såg ingen positiv effekt på avläkningen för de behandlade korna jämfört med kor i kontrollgruppen.

I sista delen av projektet undersökte vi mikrobiotan i svabbprover från juversår med hjälp av DNA-sekvensering och metagenomik och jämförde den med mikrobiotan i prover från frisk hud. Vi fann att juversår hade en lägre bakteriell diversitet än frisk hud, men att ingen specifik patogen hade samband med uppkomsten av juversår.

Samband mellan juversår och mastit analyserades och vi fann att risken för klinisk mastit var högre hos kor som fått ett kraftigt juversår, men att bakterier som vanligtvis förknippas med mastit var ovanliga i juversårens mikrobiota.

Sammanfattningsvis har detta projekt bidragit till en ökad kunskap och förståelse för epidemiologin och etiologin för juversår hos svenska mjölkkor som kan användas för att förebygga sjukdomen.

Keywords: nekrotisk dermatit, epidemiologi, etiologi, mikrobiota, sekvensering, mastit

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Dedication

To Sigge, for inspiring me with his thirst for knowledge, and to Billy, for always knowing how to make me smile.

Korna är benådade, de har en klokhet höjd över den mänskliga. En ko har alla känslor en kvinna har, och några till. De är mödrar all sin tid åt hela byns befolkning. Har man en ko så har man maten.

Aino Trosell, Hjärtblad (2010).

Contents

List of publications

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

I Ekman L, Nyman AK, Landin H, Magnusson U, Persson Waller K*. (2018). Mild and severe udder cleft dermatitis - Prevalence and risk factors in Swedish dairy herds. *Journal of Dairy Science*, 101, pp. 556-571.

II Ekman L*, Nyman AK, Persson Waller K. Incidence of udder cleft dermatitis (UCD) in dairy cows and risk factors for transitions to UCD. Submitted manuscript.

III Ekman L, Bagge E, Nyman AK, Persson Waller K, Pringle M, Segerman B. The microbiota of udder cleft dermatitis identified using shotgun metagenomic sequencing. Manuscript.

IV Ekman L, Nyman AK, Persson Waller K. Recovery from udder cleft dermatitis in dairy cows. Manuscript.

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The contribution of Lisa Ekman to the papers included in this thesis was as follows:

I Performed all herd visits, performed the statistical analyses under supervision, and wrote the manuscript with regular input from co-authors.

II Participated in planning of the study, performed all herd visits, performed the statistical analyses with input from supervisors and wrote the manuscript with regular input from co-authors.

III Participated in planning of the study, performed all field visits and samplings, performed the laboratory work, participated in bioinformatic and statistical analyses and wrote the manuscript with regular input from co-authors.

IV Participated in planning of the study, performed all field visits, performed the statistical analyses with input from supervisors and wrote the manuscript with regular input from co-authors.

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Abbreviations and definitions

16S amplicon sequencing	A method in which a specific part of the bacterial genomic material is amplified and then sequenced
CI	Confidence interval
DIM	Days in milk (days since calving)
Dysbiosis	Microbial imbalance on or inside the body
I/F	Indentation or fold (at the fore udder attachment)
k-mer	Subsequences of a biological sequence (e.g. a DNA-sequence) of the length *k*
Metagenomics	The study of genetic material recovered directly from environmental samples
Microbiota	Community of microbes within a habitat, generally including bacteria, archaea, viruses, fungi and protozoa
OR	Odds ratio
SCC	Somatic cell count
SH	Swedish Holstein breed
Shotgun sequencing	A method used for sequencing DNA with no, or very few, amplification steps before sequencing
SOMRS	Swedish official milk recording scheme (Kokontrollen)
SR	Swedish Red breed
UCD	Udder cleft dermatitis
VTCM	Veterinary-treated clinical mastitis

1 Introduction

This thesis includes epidemiological and microbiological studies on udder cleft dermatitis (UCD) in dairy cows. Although this skin condition has been mentioned in the literature for several decades, there is a lack of research on UCD, and the etiology is largely unknown. As UCD has a negative impact on animal welfare and may impair the overall udder health in dairy herds, it is important to increase the understanding of the condition and find ways of preventing and treating UCD. This thesis focuses on increasing the understanding of UCD from a Swedish perspective. The introduction gives an overview of the current knowledge of UCD and also includes a brief introduction to the subject of dermatology in dairy cows and common skin conditions, as well as a brief introduction to the dairy cattle sector in Sweden.

1.1 The modern dairy cow

Since the domestication of cattle (*Bos taurus*), around 10,000 years ago, the cow has been an important provider of food, leather, security and wealth. Through selection of cows with high milk yield, the development of the domesticated cow has led to the modern dairy cow, producing on average 10,400 kg milk/year (Växa Sverige, 2020a). Breeding for selected genetic traits and better management have enabled this remarkable development. However, the selection of high-yielding cows has led to reduced udder health and fertility, and during the early 1990's, breeding programs started to include traits other than production in the evaluation for breeding (Oltenacu & Broom, 2010). Nevertheless, mastitis (inflammation of the mammary gland), commonly caused by intramammary infection, is the most common and costly disease in the global dairy industry, as well as in Sweden (Halasa et al., 2007; Växa Sverige, 2020b).

In recent decades, there has been a structural transformation of the dairy industry in several countries, including Sweden. This transformation has led to a reduced number of dairy farms, increased herd size at the remaining farms, as well as a transition from tie-stalls to free-stalls, in which the cows live in loose housing systems. Today, there are around 3,400 dairy farms and 300,000 dairy cows in Sweden, producing 2,700,000 tons of milk per year (SBA 2020). The average number of cows per herd is 92, and the majority of the cows are of the Swedish Red (SR) and Swedish Holstein (SH) breeds.

1.2 Bovine skin and common skin conditions

The mammal skin fills many important functions and is the barrier between the body and the external environment. It protects us from physical trauma, invasion of microorganisms, sun radiation and water. Several conditions may afflict the skin, and may be caused by infectious pathogens, external influences, such as trauma or irritating substances, or internal factors, such as allergic reactions or metabolic disturbances. The following section gives an overview of the important features of the skin as well as common bovine skin conditions.

1.2.1 Bovine skin and the suspensory apparatus of the udder

The epidermis is the most superficial layer of the skin and comprises different strata including the outermost stratum corneum in which the keratinocytes are completely keratinized and ready to be worn off by friction and tear (Frandson & Spurgeon, 1992). The underlying dermis consists of connective tissue and contains nerve endings, various glands, hair follicles, blood and lymph vessels. The glands of the skin secrete sebum (sebaceous glands), sweat (sweat glands), or pheromones (specialized skin glands), and the skin is also important in the production of vitamin D.

In dairy cows, as well as in other mammals, the skin loses its elasticity and become thinner with age (Blowey & Edmondson, 2010; Coltman et al., 2017) and, in combination with other mechanisms, this lead to impaired wound healing capacity in older individuals (Lindholm, 2012). The udder base of dairy cows is covered by thin, soft and loose skin, whereas the skin of the teats is firmly established in the subcutaneous tissue (Persson Waller, 2018). The expansion of the udder during lactation causes stretching of the udder skin. Thus, the udder skin of lactating older cows is likely to be thinner than the udder skin of heifers and non-lactating cows.

The udder of the modern dairy cow may weigh up to around 75 kg and the suspensory apparatus of the udder is important in maintaining a functional and healthy udder (Blowey & Edmondson, 2010). The suspensory apparatus involves the skin and the subcutaneous connective tissue, but mainly comprises deep ligament plates on the lateral and medial sides of each udder half, as well as connective tissue fibers between the abdominal wall and the dorsal forepart of the udder (Tanhuanpää, 1995; Jalakas et al., 2000). Weakening of the suspensory apparatus is common and lead to deformation of the udder, most often resulting in a gradually lower hanging udder, referred to as increased udder depth (ICAR, 2015; Persson Waller, 2018). Ruptures of the suspensory apparatus is uncommon, but can occur gradually or sudden and be seen as a drop of the entire udder if the lateral ligaments rupture, as an enlargement of the front portion of the udder and swelling at the fore udder attachment if the anterior ligament ruptures or as outward pointing teats and loss of the udder cleft if the medial ligament ruptures (Blowey & Edmondson, 2010). As the elastic connective tissue deteriorates with age, weakening and ruptures of the suspensory apparatus is more common in older cows. Other predisposing factors are over-engorgement and edema of the udder as well as poor udder conformation (Blowey & Edmondson, 2010). Deformation of the udder due to loss of function of the suspensory apparatus could also lead to further stretching of the udder skin.

Skin microbiota

A variety of bacteria thrive on the skin surface and, in humans, different skin sites harbor different types of bacteria, depending on the presence of glands, hair and moisture (Baviera et al., 2014). Recent studies have also identified commensal bacteria in the dermis and superficial adipose tissue (Nakatsuji et al., 2013; Prast-Nielsen et al., 2019). The community of microorganisms present in an environment may be referred to as the microbiota, generally including Bacteria, Archaea, viruses, fungi and protozoa. As new technologies have enabled more extensive investigations of such communities, several important functions of the microbiota of healthy skin have become increasingly known (Grice & Segre, 2011; Baviera et al., 2014). The microorganisms of the skin are involved in immune-regulatory functions and they also produce substances that inhibit the growth of other, potentially pathogenic, microorganisms (Musthaq et al., 2018). Normal skin has acidic pH and high salt concentration, which constitute a natural selection of microbes that may inhabit the skin surface, and *Staphylococcus* and *Corynebacterium* are among the most common genera found in human skin microbiota (Fyhrquist et al., 2016). Apart from bacteria, some eukaryotic species are considered to be part of the normal skin flora in humans; these include fungal organisms (e.g. *Malassezia* spp., *Debaryomyces* spp., *Cryptococcus* spp.) and *Demodex* mites (Grice & Segre, 2011).

In dairy cows, several studies have investigated bacteria on the teat and udder skin, with a focus on udder pathogens and their presence in the cow's environment (Matos et al., 1991; Taponen et al., 2008; Braem et al., 2013) or in comparison to milk microbiota (Dahlberg, 2019). Such studies of the normal bacterial flora associated with teat skin and the teat canal yield some information about the microbiota of bovine skin, although the findings of bacteria may not be representative of other skin sites. Swab samples from the teat apices of dairy cows revealed a high bacterial diversity, mainly within four phyla: Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes (Braem et al., 2012). Commonly identified genera include *Staphylococcus, Corynebacterium, Aerococcus, Acinetobacter* and *Bifidobacterium* (Braem et al., 2012; Dahlberg, 2019). *Staphylococcus aureus* has been found in different skin sites of heifers (Matos et al., 1991) and on the teat skin of lactating cows (Zadoks et al., 2002). In a comparison of the distribution of *Staphylococcus* spp. in samples from animal and human skin, Nagase et al. (2002) found that *S. xylosus, S. sciuri* and *S. aureus* were common in samples from the backs of Holstein cows, whereas in humans, *S. epidermidis, S. warneri* and *S. hominis* were frequent findings. Farm practices, such as bedding, feed and milking hygiene, have been shown to affect the microbial community present on teat skin (Monsallier et al., 2012). Cows fed silage had higher counts of lactobacilli and yeasts than cows fed hay when swab samples from teat skin were cultured. In another study, 30 different genera of fungi were found on healthy bovine skin, of which *Nigrospora* and *Fusarium* were among the most common (do Amaral et al., 2011).

1.2.2 Common bovine skin conditions

Infectious skin conditions

The skin can be affected by several infectious agents, such as viruses, bacteria, fungi, ectoparasites, insects and ticks. Common viral skin conditions in cattle include infections by agents of the Poxviridae family, herpesvirus infections and bovine papilloma virus, the latter causing different types of warts and the other two often affecting the teats and udder, causing vesicles and blisters with varying degrees of pain and discomfort (Scott, 2007).

As bacteria are part of the normal skin flora, and are abundant in the cow's environment, they are found in different skin conditions, as well as on healthy skin. Breakage of the protective barrier of the skin, irrespective of the cause, predisposes to bacterial overgrowth, and secondary bacterial infections are common, for example, after traumatic injuries or viral infections. The bacterial involvement in the primary disease can, however, sometimes be hard to evaluate as the same bacteria could be present in the environment and on healthy skin (Jackson, 1993). Superficial staphylococcal infection, impetigo, may affect the teats and udder of dairy cows, causing pustules, erosions and crusts (Scott, 2007). Furunculosis, folliculitis and rain scald (dermatophilosis) are other superficial skin conditions caused by bacteria, all dependent on the breakage of the protective barrier of the skin that may appear anywhere on the body (Scott, 2007). *Fusobacterium necrophorum* may infect damaged teat apices and cause necrotic lesions, so called "black spots" and may also cause infections that appear anywhere on the body (though most common in skin folds, such as axillae, groin or udder cleft) and cause a foul-smelling necrotic dermatitis (Scott, 2007).

Digital dermatitis and interdigital phlegmon are two common bacterial conditions associated with the hooves of cattle, but they primarily affect the skin surrounding the claws (Bergsten, 1997). Interdigital phlegmon affects the interdigital skin and the opportunistic anaerobe *Fusobacterium necrophorum* is frequently found in these lesions. Interdigital phlegmon lesions develop when there is a local injury to the skin, due to trauma, uneven ground, rough flooring or maceration of the skin by water, faeces or urine. Digital dermatitis is a highly contagious condition that affects the epidermis proximal to the coronary band (Bergsten, 1997). The etiology of the condition is believed to be multifactorial and involves several bacterial agents including *Treponema* spp., which appear to be important in the development of digital dermatitis (Pringle et al., 2008). In a deep sequencing analysis Krull et al. (2014) monitored the microbiotic changes in biopsies from interdigital skin of cattle as digital dermatitis lesions developed. They found that the family Spirochaetaceae increased most in abundance in the digital dermatitis lesions compared to the microbiota of healthy skin, but that Mycoplasmataceae, Moraxellaceae and Porphyromonadaceae also increased in early-stage lesions.

A common fungal skin infection in cows is dermatophytosis (ring worm), most often caused by *Trichophyton verrucosum*. The condition is manifested by typical circular, hairless, grey lesions (Bond, 2010).

Different types of mange mites may cause more or less pruritic skin disorders in cattle of all ages (Scott, 2007). The most common mite in Swedish herds is

Chorioptes bovis which cause lesions and itching around the tail head, perineum and caudal udder (SVA, 2019). Lice, insects and ticks may also cause itching, skin irritation and/or inflammation (Fadok, 1984; Jackson, 1993).

Non-infectious skin conditions

Several environmental conditions may affect the skin, such as sunburn, frost injuries and contact dermatitis due to irritating substances or hypersensitivity of the skin. Symptoms vary in intensity and may include erythema, edema, papules, scales, vesicles, erosions, ulcers, necrosis and crusts (Scott, 2007). Self-trauma (by licking or scratching) or secondary infections may worsen the condition. As the skin has a limited number of ways of responding to damage, the cause of dermatitis or a skin injury is not always clear, but the distribution of the lesions may offer an indication of the cause (Scott, 2007).

Traumatic injuries can affect any body site of a dairy cow, but the teats and udder are in an exposed position and may be particularly vulnerable to such injuries. Environmental factors such as cubicle fittings or thorny shrubbery at pasture can be risk factors, but there are also other factors to consider. A cow with a deep udder and reduced mobility (due to lameness or disease) is also at high risk of sustaining traumatic teat or udder injuries (Scott, 2007).

Hock lesions are extremely common in dairy cows housed in free-stalls (Weary & Taszkun, 2000; Potterton et al., 2011a; Ekman et al., 2018) and their etiology is probably both environmental and affected by internal factors. The lesions may develop following prolonged high local pressure on hard surfaces or edges, or by abrasive lying surfaces (i.e. repeated physical conflicts between the cow and her housing environment (Brenninkmeyer et al., 2013). There are different manifestations of hock lesions often categorized as hair loss, ulceration and swelling (Laven & Livesey, 2011). Studies have found different risk factors for different manifestations, which suggest different etiologies and pathogenesis depending on the manifestation (Potterton et al., 2011b; Ekman et al., 2018).

Udder-thigh dermatitis affects the medial thigh and the side of the udder. It is more common in primiparous cows, and udder edema is a predisposing factor (Roy et al., 2012). The lesions can be uni- or bilateral and usually present with initial erythema and swelling, followed by necrosis and sloughing of skin layers (Sigmund et al., 1983). *Fusobacterium necrophorum* is a common finding in udder-thigh dermatitis lesions (Roy et al., 2012).

In contrast to udder-thigh dermatitis, UCD is located at the fore udder attachment or between the udder halves and is more common in multiparous cows (Persson Waller et al., 2014). Further information on UCD is given in the next section.

Other non-infectious skin disorders include nutritional disorders (e.g. zinc-responsive dermatitis and photodermatitis), different types of intoxications (e.g. iodism), immunological skin disorders (e.g. urticaria) and neoplastic growths (Scott, 2007).

1.3 Udder cleft dermatitis

Udder cleft dermatitis in dairy cows is an inflammatory skin condition most often located in the anterior junction between the udder and the abdominal wall. The condition is also known as ulcerative mammary dermatitis, necrotic dermatitis, intertrigo or udder sores and has been reported in several countries (Warnick et al., 2002; Persson Waller et al., 2014; Riekerink et al., 2014). The UCD lesions range from small, eczema-like skin changes to deep, exudative lesions. The lesions impair the welfare of the affected cows and may also become a hygiene issue in the dairy herd, as exudations of pus and blood are common and could contaminate milking equipment or spread bacteria between cows (Beattie & Taylor, 2000).

1.3.1 Clinical presentation

The UCD lesions can be classified into mild or severe according to their appearance, although the nomenclature may vary between studies (Persson Waller et al., 2014; Riekerink et al., 2014; Bouma et al., 2016). Mild UCD is commonly defined as small crusts, papules or pustules, often in combination with red and thickened skin and serum exudation, whereas severe lesions include large crusts and a breach of skin integrity, often with blood, pus, necrotic tissue and a foul odor (Persson Waller et al., 2014; Bouma et al., 2016). The lesions may develop into deep crater-like ulcers, and some severe cases also present with proliferations of the skin. Examples of mild and severe UCD lesions are presented in Figure 1.

The most common location of UCD is at the fore udder attachment, close to the ventral mid-line of the cow, where the udder base meets the abdominal wall. The lesions may also be located between the front quarters of the udder, and sometimes cover the entire median sulcus of the udder as well as the fore udder attachment.

UCD rarely cause general clinical symptoms in the cow, such as fever or inappetence.

1.3.2 Prevalence, incidence and duration

Prevalence

Studies on UCD have been published from the UK (Boyer & Singleton, 1998), the USA (Warnick et al., 2002), Sweden (Persson Waller, 2003), Denmark (Hansen & Nissen, 2010), The Netherlands (Riekerink et al., 2014) and Norway (Eikeland & Paulsen, 2018). However, only two studies have investigated the prevalence of UCD in randomly selected dairy herds (Persson Waller et al., 2014; Riekerink et al., 2014). In these two studies, the average within-herd prevalence of UCD was 18% (range 0 – 39%; Persson Waller et al., 2014) and 5% (range 0 – 15%; Riekerink et al., 2014). To better understand the distribution of UCD within and between countries, further prevalence studies of UCD are warranted.

Incidence and duration

Only one published study has investigated the incidence and duration of UCD. In this longitudinal Dutch study, it was found that the incidence of UCD was 1.94 cases per 100 cow-weeks at risk and that the median observed duration of UCD was 16 weeks (Bouma et al., 2016). The high incidence in combination with the long duration may explain the high prevalence seen in some herds. Further longitudinal studies of UCD are warranted to increase the understanding of the clinical course of UCD.

1.3.3 Etiology

The etiology of UCD is largely unknown. Although certain pathogens, such as mange mites (Allenstein, 1991), *Treponema* spp. (Boyer & Singleton, 1998) and bovine herpesvirus (Lyman, 2019), have been proposed as potential contributors to the development of UCD lesions, their role in the etiology is unclear. Other studies indicate a multi-factorial, non-contagious origin of UCD, although the involvement of specific microbes cannot be ruled out (Persson Waller et al., 2014; Riekerink et al., 2014; van Werven et al., 2019).

Figure 1. Illustrations of udder cleft dermatitis (UCD) lesions of score 0 – 5, also defined as mild and severe UCD. Photo: Lisa Ekman

Microbial investigations

Hoard's Dairyman published a letter in 1991 in which Allenstein suggested that mites (i.e. *Chorioptes bovis)* were the cause of "smelly udders" and reported that treatment of these parasites improved the healing of UCD lesions. Since then, several studies have investigated the relationship between mange mites and UCD but none of them found significant evidence of the involvement of mites in the pathogenesis of UCD (Warnick et al., 2002; Hansen & Nissen, 2010; Persson Waller et al., 2014).

In 1998, Boyer and Singleton reported that outbreaks of superfoul (a peracute form of digital dermatitis) coincided with subsequent outbreaks of severe UCD in two dairy farms in the UK. They discussed how the clinical presentation of UCD sometimes resembles that of digital dermatitis and that the same bacteria might be responsible for both conditions. Several subsequent investigations of the involvement of *Treponema* spp. in the pathogenesis of UCD have been performed, as certain *Treponema* spp. are associated with digital dermatitis. Beattie and Taylor (2000), however, found evidence of *Treponema* spp. in only four out of 13 samples from UCD lesions, and more recent studies also failed to find evidence of treponemes as a cause of UCD (Hansen & Nissen, 2010; Bengtsson, 2013). Moreover, Warnick et al. (2002) found that cows that had previously been diagnosed with digital dermatitis had a lower risk of UCD than other cows. Through specific PCR investigations of biopsies of UCD lesions, with amplicons targeting *Treponema* spp., two studies found different species of *Treponema* in UCD lesions, some of which are also associated with digital dermatitis (Stamm et al., 2009; Evans et al., 2010). Evans et al. (2010) also performed immunohistochemistry on biopsies from UCD lesions and in one of these biopsies, high numbers of treponemes were seen invading the epidermis. However, the bacteria were only found in three out of eight samples and were thus not present in all samples.

Less specific microbiological investigations of UCD have been performed, mainly by culturing material from swab samples or through histopathological examination of biopsies from UCD lesions. Persson Waller (2003) performed aerobic and anaerobic bacterial culturing from swab samples from three cases of severe UCD. *Corynebacterium* spp., *Trueperella pyogenes* and *Fusobacterium necrophorum* were present in all samples. In addition, a biopsy from one of these cases revealed hyperkeratosis, vesicle formation and infiltration of inflammatory cells, such as neutrophils and eosinophil granulocytes.

Similar investigations were performed by Warnick et al. (2002), with anaerobic culturing of swab samples and histological examinations of biopsies from UCD lesions. Bacterial findings included *Fusobacterium necrophorum* and *Bacteroides* spp., while histological findings included perivascular-to-interstitial dermatitis. Beattie and Taylor (2000) performed aerobic and anaerobic culturing of swab samples and found a variety of bacteria (e.g. *Corynebacterium* spp, *Trueperella pyogenes* and *Staphylococcus* spp.), as well as some fungal species, such as *Geotrichum* spp. and *Candida* spp.

A more recent study used 16S rRNA-amplicon sequencing to map the bacterial metagenome of UCD lesions and compared it with that of healthy skin (Sorge et al., 2019). They found that certain bacterial genera were more common in samples from UCD lesions, such as *Fusobacterium* spp., *Helcococcus* spp., *Anaerococcus* spp., *Prevotella* spp., *Trueperella* spp. and *Porphyromonas* spp., compared to samples from healthy control cows. They also found that the bacterial diversity was higher in control samples compared to samples from UCD lesions. Sorge et al. (2019) also investigated skin biopsies from UCD lesions, as well as from the fore udder attachment of healthy control cows and found serocellular crusts, haemorrhage, inflammatory cells, ulceration and erosion of the epidermis, as well as edema, degeneration and necrosis in the dermis of biopsies from UCD lesions, indicating a severe inflammatory process. Histological signs of haemorrhage were also found in two of the biopsies from healthy control cows.

Herd- and cow-related risk factors

Epidemiological studies have identified cow- and herd-related risk factors associated with UCD, most of which are related to the prevalence of UCD as most previous studies investigating risk factors are cross-sectional.

At herd level, factors that have been associated with UCD are breed, herd production and the use of a footbath. Persson Waller et al. (2014) found that herds with a high proportion (≥50%) of SR cows and herds with a high average milk production (≥10,900 kg/cow and year) had a higher UCD prevalence compared to herds with a high proportion of SH cows, and less than 20% SR cows and herds with a lower production level (<10,000 kg/cow and year). In a Dutch study, a high herd mean production (>9,000 kg/cow and year) was also found to increase the risk of UCD, as did the use of a footbath (Riekerink et al., 2014). The authors discussed that the use of a footbath might be an indicator of hoof-related herd problems, and that the number of herds in the study was too low to draw any firm conclusions.

Several cow-related risk factors for UCD have been identified, including breed, days in milk (DIM), milk yield, parity and udder conformation. A Swedish study found that SR cows and crossbreeds had a higher risk of UCD than SH cows (Persson Waller et al., 2014). A higher risk for UCD in cows later in lactation has been identified (Warnick et al., 2002; Hansen & Nissen, 2010), although this association was only seen for first parity cows in one of these studies (Warnick et al., 2002). Persson Waller et al. (2014) also found that higher milk yield was positively associated with UCD. In addition, a higher risk for UCD was seen in cows with three or more lactations compared to younger cows (Warnick et al., 2002; Persson Waller et al., 2014; Riekerink et al., 2014). Certain udder conformation traits affect the risk of UCD, such as the conformation of the fore udder attachment, in which a small angle between the udder and the abdominal wall (loose fore udder attachment) increased the risk of UCD (Hansen & Nissen, 2010; Riekerink et al., 2014) and a strong fore udder attachment (wide angle between the udder and the abdominal wall) decreased the risk (Persson Waller et al., 2014). In addition, a deep udder increased the risk of UCD (Hansen & Nissen, 2010; Riekerink et al., 2014), as did larger front quarters (Riekerink et al., 2014), whereas a strong median suspensory ligament increased the risk in one study (Hansen & Nissen, 2010), while no association was seen in another study (Riekerink et al., 2014).

Most previous studies are cross-sectional, but Bouma et al. (2016) performed a longitudinal study and investigated the risk factors associated with the incidence of UCD. They found that the incidence was higher in cows in third or higher parity as well as higher in cows with more DIM.

The risk factors investigated in a previous Swedish study only explained a small part of the variation in UCD prevalence (Persson Waller et al., 2014; Riekerink et al., 2014) indicating that other factors of importance for UCD are yet to be identified. Moreover, as the degree of UCD severity differs between cows, analyses of separate risk factors for mild and severe UCD are warranted. For example, risk factors for hock lesions in dairy cows have been shown to vary depending on the severity of the lesion (Potterton et al., 2011b).

1.3.4 Recovery from UCD and treatment regimes

As previously mentioned, UCD lesions often have long duration and delayed healing. Bouma et al. (2016) found that mild lesions are more likely to heal than severe ones, but knowledge on the spontaneous recovery rate and which factors affect recovery is sparse. Such knowledge is important to increase the understanding of the clinical course of UCD, and to identify cows with the highest – as well as those with the lowest – chance of spontaneous recovery.

There is currently a lack of effective treatment strategies for UCD in Sweden, something that is warranted by both farmers and veterinarians. According to anecdotal reports treatment strategies for severe UCD include washing with soap and iodine, and the use of topical antibiotics or other compounds with antibacterial and skin softening effects (Allenstein, 1991; Boyer & Singleton, 1998; Persson Waller, 2003).

A recent Dutch study tested two different topical treatments on UCD (van Werven et al., 2018). In the study, mild lesions were treated with a non-alcoholic film layer, whereas severe lesions were treated with an enzyme alginogel. Both treatments were derived from treatment strategies for human chronic wounds. They found that the alginogel significantly improved the healing of severe lesions, whereas no effect was seen for the treatment of mild UCD (van Werven et al., 2018). Enzyme alginogels have been shown to have several positive effects on wound healing, such as continuous wound debridement, epithelial-stimulating and antibacterial effects (Beele et al., 2012). In Sweden, no alginogel products approved for bovine wound-treatment are available on the market. There are, however, other products with similar properties. A topical treatment containing chelated copper and zinc proved to be efficient for the treatment of digital dermatitis (Holzhauer et al., 2011; Relun et al., 2012) and also had a positive effect on the recovery from several cases of severe UCD, although these results were from a pilot study and not from a controlled clinical trial (Lammers et al., 2017). According to the manufacturer of the topical treatment, zinc enhances wound recovery and stimulates the growth of epithelial cells while copper has antimicrobial effects and stimulates the formation of new blood vessels (Lammers et al., 2017). Studies that evaluate treatment strategies for UCD that are implementable under Swedish conditions are warranted.

1.3.5 Potential consequences of UCD

It is not known if and how UCD affects the overall health, milk production and sustainability of dairy cows.

The fact that UCD lesions contain a variety of bacteria (Warnick et al., 2002; Persson Waller, 2003) indicates a possible link between UCD and infectious diseases such as mastitis. In line with this, Persson Waller et al. (2014) found an around three times higher risk for veterinary-treated clinical mastitis (VTCM) in cows with UCD compared to cows without UCD. The causal relationship is not clear, but it is possible that UCD serves as a reservoir for udder pathogens although it is not well known whether such pathogens are a common finding in the UCD microbiota. It is also possible that UCD and mastitis share common risk factors.

Other reported consequences of the more severe cases of UCD are severe bleeding, sometimes with laceration of the mammary vein and subsequent death (Hansen & Nissen, 2010), as well as embolic pneumonia (Millar et al., 2017). These findings emphasize the importance of the prevention and effective treatment of UCD.

1.4 Comparative aspects

UCD lesions share certain features with skin conditions in other species, such as intertrigo (mostly reported in humans and dogs), different types of human chronic wounds such as pressure ulcers, venous leg ulcers and diabetic foot ulcers, and shoulder ulcers in sows. In these conditions, delayed healing and chronicity are often seen. In addition, the clinical appearance of these conditions often resembles the clinical appearance of UCD lesions.

1.4.1 Intertrigo

Intertrigo is a skin fold dermatitis that can occur in several species (including humans). It can be defined as a dermatitis that develops because of friction between opposing skin surfaces (e.g. adjacent folds) where air circulation is limited. Intertrigo is not a common condition in cows, but similarities between intertrigo and UCD have been proposed (Beattie & Taylor, 2000). Udder-thigh dermatitis in dairy cows could possibly be viewed as a form of intertrigo. However, udder-thigh dermatitis is closely related to udder edema and swelling of the udder, and probably has a different etiology than intertrigo (Roy et al., 2012).

The presence of extensive skin folds leads to skin-on-skin friction and impairs air circulation, which facilitates a moist environment that damages the skin barrier and predisposes to bacterial colonization or skin infection (Patel, 2009; Kalra et al., 2014). Intertrigo lesions are characterized by initial mild erythema that may progress to a more intense inflammation with erosions, oozing, exudation, maceration, necrosis and crusting (Janniger et al., 2005). Bacteria commonly associated with intertrigo in humans are *Staphylococcus aureus, Streptococcus* spp.*, Pseudomonas aeruginosa, Proteus mirabilis, P. vulgaris* and some fungal species, e.g. *Candida* spp (Janniger et al., 2005). In dogs, *Staphylococcus pseudintermedius* is a common part of the normal canine mucosal flora, and it is also frequently found in superficial pyoderma caused by intertrigo (Bannoehr & Guardabassi, 2012; Ravens et al., 2014). Other bacteria that may cause pyoderma due to intertrigo in dogs are *Staphylococcus aureus* and *Staphylococcus schleiferi,* and opportunistic bacteria, such as *Escherichia coli* and *Proteus* spp., may worsen existing skin diseases (Patel, 2009). Treatment strategies for intertrigo include reducing moisture and friction in the area, as well as the use of antibacterial or antifungal products, depending on the specific condition (Janniger et al., 2005).

1.4.2 Chronic human ulcers

Pressure ulcers, venous leg ulcers and diabetic foot ulcers are three types of common chronic human wounds. Although their etiology differs, their impact on the quality of life of patients and health care costs are similar.

Pressure ulcers can be defined as "an area of localized damage to the skin and underlying tissue caused by pressure, shear, friction or a combination of these" (Keller et al., 2002, p.1380). A compression of soft tissues between bony prominences and the underlying surface causes occlusion of blood vessels and a lack of oxygen in the tissues, leading to cell death and ulcer formation. Moisture (due to e.g. diarrhea, incontinence or sweating), lack of mobility and poor nutritional status increase the risk of patients developing pressure ulcers (Keller et al., 2002).

Venous leg ulcers occur secondary to venous insufficiency and increased venous pressure, leading to extrusion of polysaccharides and fibrinogen from blood vessels into the surrounding tissue (Lindholm, 2012). This results in a localized inflammatory process in the tissue, which may develop into ulceration of the skin.

The etiology of diabetic foot ulcers is complex but is mainly related to damage to the peripheral nerves, diabetic neuropathy and inadequate arterial blood flow (Margolis et al., 2002).

Several studies have investigated the bacteria present in human chronic wounds (Dowd et al., 2008; Gardiner et al., 2017; Johnson et al., 2018). Many of the identified bacteria are also common findings in healthy skin and even though chronic ulcers are always colonized with bacteria, they are not necessarily infected (Lindholm, 2012). Although the microbiota differ between wound types and individual samples, common findings include *Corynebacterium* spp., *Staphylococcus* spp., *Pseudomonas* spp, and *Anaerococcus* spp. (Dowd et al., 2008; Smith et al., 2010). In addition, Dowd et al. (2008) found a more anaerobic microbiota in pressure ulcers compared to the other two wound types.

Treatment strategies for human chronic ulcers include the identification and regulation of potential underlying causes (e.g. relieving pressure by repositioning or treatment of underlying diseases), debridement to remove dead tissue, and creating a favorable environment for wound healing (Vowden et al., 2008; Lindholm, 2012). Such a favorable environment can be achieved by humectant treatment to avoid drying out, management of potential wound infection and promoting sufficient blood flow to the wound bed. An aggravating circumstance in the recovery from these types of ulcers is biofilm, which is produced by certain bacteria (Wu et al., 2019). Biofilm is a complex polymicrobial community that can attach to surfaces and secrete extracellular polymeric substances (Suleman & Percival, 2015). This secretion, and other synergistic effects among the bacteria in the biofilm, protect the microbial community, and biofilms can withstand both physical and chemical products, such as antibiotics. *Pseudomonas* spp., *Staphylococcus* spp. and *Prevotella* spp. are a common finding in biofilms in human chronic ulcers (Dowd *et al.*, 2008; Wolcott et al., 2016).

1.4.3 Shoulder ulcers in sows

Hard flooring in pig stables may lead to sows lying on hard surfaces. In similarity to hock lesions in dairy cows and human pressure ulcers, this may lead to lack of oxygen in the skin and underlying tissues and thus, development of shoulder ulcers (Herskin et al., 2011). The ulcers can be superficial or involve deeper tissues, sometimes even the underlying scapular bone is affected. The development and pathogenesis are believed to be similar to the development of human pressure wounds, and pigs have been used as animal models for human ulcers (Herskin et al., 2010). Lund (2003) performed aerobic culturing of samples from shoulder ulcers. The species most commonly found was *Trueperella pyogenes,* but *Staphylococcus aureus,* β-hemolytic streptococci and some other bacteria were also cultured (Lund, 2003, see Karlsson, 2014). Karlsson et al. (2014) investigated the role of *Treponema* spp. in porcine skin ulcers and found that treponemes were present in the majority of the investigated shoulder ulcers and that they were more common in severe ulcers compared to mild ones.

2 Aims of the study

The overall aim of this PhD project was to increase the understanding of the epidemiology and etiology of UCD in Swedish dairy cows, and to improve recommendations on prevention and treatment regimens.

Specific aims were:

- To assess the prevalence of UCD in Swedish dairy cattle herds.
- To identify cow- and herd-related risk factors associated with the prevalence of mild and severe UCD.
- To investigate the incidence and duration of, as well as recovery from, UCD and to identify cow-related factors associated with transitions to UCD, and with recovery from UCD.
- To compare the microbiota of mild and severe UCD lesions with that of skin at the same body site in healthy control cows and investigate whether certain microorganisms are associated with UCD.
- To investigate associations between UCD and mastitis and culling.
- To evaluate the effect of a topical spray in the treatment of UCD under field conditions.

3 Materials and Methods

A summary of the materials and methods used in papers I – IV is given in this section. Detailed descriptions can be found in the corresponding sections within each paper. An overview of the performed studies is presented in Figure 2.

3.1 Study populations and study designs

In paper I, 99 Swedish dairy herds were enrolled in a cross-sectional prevalence study to investigate the prevalence of mild and severe UCD and associated risk factors, as well as associations between UCD and udder health and culling. Inclusion criteria were free-stall housing, tandem or fishbone milking parlor, a herd size of 50 – 210 cows and affiliation with the Swedish official milk recording scheme (SOMRS). The herds were randomly selected to represent the dairy herds in southern and central Sweden, where 90% of the herds meeting the inclusion criteria were situated. In total, 3,479 cows were included in the study.

In papers II, III and the first part of paper IV, seven of the herds from paper I were enrolled in a one-year longitudinal study to investigate incidence and duration (paper II) as well as recovery (the first part of paper IV) from UCD and associated risk factors and associations with udder health and hock lesions. Moreover, the microbiota of recently developed UCD lesions and healthy skin was also investigated (paper III). The seven herds had a UCD prevalence of 15 – 60% according to paper I, and were situated within two hours driving distance of Uppsala, Sweden, in order to facilitate herd visits. The herds were visited nine times each, at six-week intervals. Throughout the longitudinal study, 6,221 observations of 1,106 individual cows were performed.

For the second part of paper IV, four of the seven herds were enrolled in a treatment study investigating the effect of a topical spray containing chelated copper and zinc as treatment for UCD. The treatment study started shortly after the completion of the longitudinal study. It included 125 cows and continued for eight weeks with follow-up visits on days 14, 28 and 56.

All participating farmers signed a consent form to be part of the respective studies and agreed to share their herd and cow data in the SOMRS for study purposes.

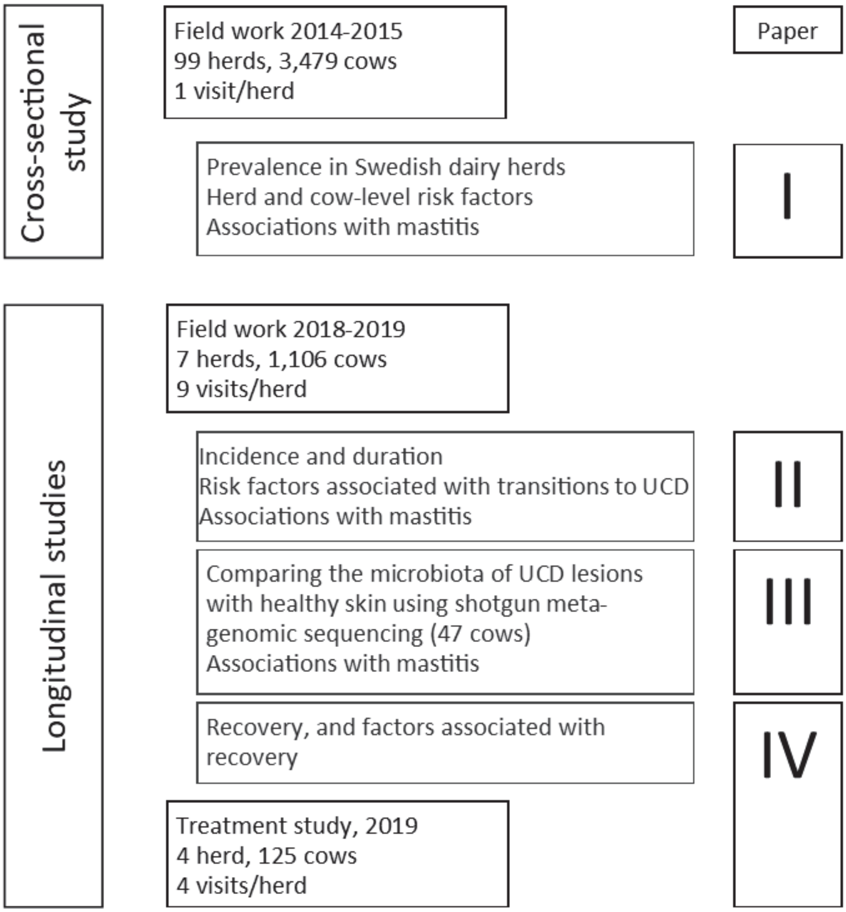

Figure 2. Overview of the studies performed between 2014 and 2019 during the course of this thesis work, with the aim to increase the understanding of udder cleft dermatitis (UCD) in Swedish dairy cows.

3.2 Herd visits and additional data collection

3.2.1 Herd-related data collection

General information on the participating herds was obtained from the SOMRS, such as herd size, production system, average milk production as well as herd health key performance indicators. In addition, during the cross-sectional study, the farmers were interviewed using a pre-designed questionnaire regarding management routines (e.g. milking, pasture, hoof trimming, cleaning, use of bedding material etc.), health status, and if and how they treated UCD lesions. On-farm observations of milking procedures, type of stable, and cubicle measurements were also performed during the cross-sectional study.

3.2.2 Cow-related data collection

Cow observations in both the cross-sectional (paper I) and the longitudinal study (papers II and IV) included scoring of UCD as well as hygiene, hock lesions and udder conformation traits. All herd visits took place during milking and cows were examined in the milking parlor, just before, during, or just after milking. In all papers, the area of the fore udder attachment was examined with a flashlight and a hand-held mirror. In many cases, the presence of UCD was hard to verify simply by looking at the fore udder attachment, as hair and skin folds could conceal skin changes in the area. Thus, in most cases palpation was also used. Disposable gloves were used at all times and were changed between examinations. For paper I, every second to third cow entering the milking parlor was examined, whereas in the other studies, all cows milked in the parlor were examined.

Scoring of UCD lesions

In paper I, the cows were registered as having no UCD (i.e. no palpable skin changes), mild UCD (i.e. hyperkeratosis, small papulae/pustulae, small crusts or serum transudation) or severe UCD (i.e. large crusts, pus, deep skin wounds, or proliferations).

In the longitudinal study, UCD lesions were scored from 0 to 5 (Table 1), where 2 corresponded to mild UCD, and 3 – 5 to severe UCD in the previous study, whereas score 1 was very mild skin changes that would not have been registered as UCD in paper I (see also Figure 1).

Table 1. *Scoring of udder cleft dermatitis (UCD) used in papers I – IV (see also Figure 1)*

UCD score (paper I)	UCD score (papers II – IV)	Clinical appearance at the fore udder attachment or between the front quarters
No UCD	0	No signs of UCD
No UCD	1	Very mild signs of UCD, such as redness of the skin, or single papule or pustule in the area of the fore udder attachment.
Mild UCD	2	Small papules/pustules or crusts, in combination with one or several signs of score 1, total size of crusts < 5 x 5 cm.
Severe UCD	3	Large crusts, with or without signs of score 1–2, total size of crusts ≥ 5 x 5 cm. No open wound.
Severe UCD	4	Open wound, with or without signs of score 1–3, total size of wound and surrounding crusts < 5 x 5 cm.
Severe UCD	5	Open wound, with or without signs of score 1 – 3, total size of wound and surrounding crusts ≥ 5 x 5 cm.

Scoring of udder conformation traits, hygiene and hock lesions

In paper I, three udder conformation traits were registered: fore udder attachment (angle between fore udder and abdominal wall), udder balance (depth of the rear udder in relation to depth of the front udder) and presence or not of an indentation or skin fold (I/F) at the anterior junction between the udder and the abdominal wall. The same udder conformation scoring was used in papers II and IV, except for udder balance which was replaced by registration of udder depth.

The cow hygiene was scored based on the cleanliness of the udder and the hind limb above the hock on the side visible from the operating area of the milking parlor. Presence of hock lesions on the same side was scored as no, mild (hair loss), or severe (skin wound or evident swelling) in paper I, whereas in papers II and IV, a more detailed scoring of hock lesions was used, which also accounted for the size of the lesions.

Cow data from the SOMRS and farmer registrations

Individual cow data on breed, parity, calvings and information from test milkings were obtained from the SOMRS. Test milking results included information on individual milk yield (kg/day), cow composite milk somatic cell count (SCC), and milk urea concentrations at test milkings within 34 days before or after the visit in paper I and within 31 days prior to the visits in papers II and IV. Registrations of veterinary-treated diseases and results from hoof trimmings were collected from the SOMRS when available, as well as information on culling.

Apart from the SOMRS records of veterinary-treated diseases, in the longitudinal study, the participating farmers were also asked to register veterinary treatments of cows during the study period.

3.2.3 Microbiological sampling and laboratory methods

In paper III, during visits 3 – 7 of the longitudinal study, recently developed UCD lesions were identified and sampled. The criteria for sampling was a cow with no UCD at the previous observation that received a UCD score of 2 or higher, or a cow with a UCD score of 2 at the previous observation that received a UCD score of 4 – 5. For every sampled lesion, the aim was to sample the skin of a control cow with no UCD from the same body site. Additional samples were also obtained from cows with "old" UCD lesions in order to obtain approximately 10 samples per category (no UCD, mild UCD, and severe UCD) from each herd. The area for sampling (UCD lesion and adjacent skin, or healthy skin at the fore udder attachment for control cows) was rubbed around 20 times using a sterile sponge pre-moistened with saline.

In total, 184 swab samples were obtained. DNA extraction was performed, and 49 samples were chosen for shotgun sequencing, including 13 samples from healthy control cows, 17 from mild UCD and 19 from severe UCD. Sequencing was performed at the Science for Life Laboratory on the SNP&SEQ Technology Platform (Uppsala, Sweden), using the Illumina NovaSeq6000 system.

3.2.4 Treatment study

In paper IV, a treatment study was performed, testing a topical spray containing chelated copper and zinc. The herds were visited four times each during the study period, on days 1, 14, 28 and 56. On day 1, all UCD lesions were thoroughly cleaned, measured, and scored. Cows were assigned into a treatment or control group. Cows in the treatment group were treated once daily for 14 – 28 days. Cows in the control group received no treatment after the initial cleaning day 1. All cows in the study were scored for UCD at the follow-up visits on days 14, 28 and 56.

3.3 Data editing and statistical analyses

Handwritten protocols from on-farm registrations were transferred into Excel worksheets. For papers I, II and IV, Stata (StataCorp LP, College Station, TX) was used for all statistical analyses. In papers II and IV, a UCD case was defined as one or more consecutive observations of UCD. In papers II – IV, UCD score 2 was re-classified as mild UCD and UCD score 3 – 5 re-classified as severe UCD to better correspond with previous studies and to avoid subgroups with too few cows.

3.3.1 Prevalence, incidence, duration and recovery

In paper I, the prevalence of mild, severe, and any type of UCD lesions among all examined cows, and within each herd, was calculated. In paper II, the prevalence of mild and severe UCD within each herd at each herd visit was calculated. In addition, the number of transitions in all directions, between healthy, mild, and severe UCD was calculated.

In paper II, the overall incidence rate of observed new cases of any type of UCD was defined as the number of new observations of UCD since the last visit divided by the total "cow-time-at-risk" for the same time period, standardized into new cases per cow-year at risk.

A case that was preceded and followed by at least one observation of no UCD was defined as a case with known start and end, whereas other cases were defined as cases with unknown start and end. The estimation of the observed duration was based on the observed number of consecutive observations of UCD and the visit intervals. The duration of cases of only mild UCD observations was compared with the duration of cases including severe UCD using the Wilcoxon rank-sum (Mann-Whitney) test.

In paper IV, recovery was defined as at least two consecutive observations of no UCD after a UCD case. The proportion of cows that recovered was calculated, as well as the proportion of cows that had recurrent cases of UCD after the recovery.

3.3.2 Risk factor analyses

In paper I, associations between the presence of any type of, mild, and severe UCD and explanatory variables were analysed in separate mixed-effect univariable and multivariable logistic regression models, including herd as random factor. A final, multivariable mixed-effect model for each outcome (any type of, mild, and severe UCD) was then built, including the variables that were significant ($P \leq 0.05$).

In paper II, risk factors for a transition from being unaffected to having any type of, mild, or severe UCD were also analysed using mixed-effect univariable and multivariable logistic regression analysis, with herd and cow included as random factors and a transition to UCD (yes or no) as a binary outcome.

In paper IV, factors associated with recovery from UCD were analysed using discrete-time survival analyses. Two separate models were used, one for UCD cases for which the start of UCD was known, and one where all UCD cases were included, including cows that had UCD at the start of the study as well as cows that developed UCD during the study period.

3.3.3 Comparison of microbiota in UCD lesions and on healthy skin

In paper III, data from the shotgun sequencing were subjected to bioinformatic analyses, including comparing *k*-mer frequencies, filtering of bovine DNA and *k*-mer based classification of bacterial, archaeal, viral, fungal and protozoan DNA reads present in the samples. In an additional analysis, the sequenced reads were compared with the genome of the itch mite, *Sarcoptes scabiei.*

The number of reads assigned to different taxa within each sample was normalized to a proportion of the total number of classified reads for that specific sample. Data dimensionality reduction with principal component analysis (PCA) was performed to see if and how samples differed from each other. The overall abundance of different microbes within samples and sample types (control, mild, and severe UCD) was described. The Wilcoxon rank sum (Mann-Whitney) test was used to compare the abundance of different taxa between different sample types, using the Bonferroni method to adjust for multiple testing. The bacterial alpha-diversity (diversity within samples) was investigated by calculating the Shannon diversity index, and the results were compared between sample types using the Wilcoxon rank sum test.

3.3.4 Analyses of the effects of UCD on udder health and culling

In paper I, associations between mild and severe UCD lesions and udder health and culling were also investigated. For this analyses, we used univariable, mixed-effect logistic regression or mixed-effect linear regression analyses, investigating mild and severe UCD as explanatory variables for three cow-level outcomes: 1) milk SCC; 2) VTCM within 90 days before or after the visit; and 3) culling of the cow within 90 days of the visit.

In paper II, similar associations were investigated, but the focus was on potential consequences of a recently developed UCD lesion. Thus, only cows with no UCD at the previous observation were included in the analyses. We used univariable, mixed-effect logistic regression analyses with three different binary outcomes: 1) high milk SCC; 2) VTCM within approximately six weeks of the herd visit; and 3) presence of a hock lesion, registered at the same time as the UCD observation. In three different models per outcome, a recently developed UCD lesion of any type of UCD, mild UCD, and severe UCD was tested as explanatory variable for each outcome.

In paper III, bacterial species commonly associated with mastitis and that represented at least 1% of the bacterial reads in at least one sample were described, and their abundance was compared between control samples and mild and severe UCD samples.

3.3.5 Evaluation of topical treatment of UCD

In paper IV, an ordered logistic regression model was used to analyze the effect of treatment on the UCD status on day 56 compared to day 1. The outcome was no, mild or severe UCD at the last follow-up visit (day 56) in the treatment trial, with treatment (yes or no) as an explanatory variable. Factors with a potentially confounding effect on the outcome, such as breed, herd, parity and duration of lesion before the start of the treatment study were also included in the analyses.

4 Results

This section gives an overview of the most important results in papers I – IV. Detailed information of the results can be found in the corresponding sections within each paper.

4.1 Prevalence, incidence, duration and recovery (I-II, IV)

The overall prevalence of UCD in paper I was 28% and the proportion of cows with mild and severe UCD was 19% and 9%, respectively. The within-herd prevalence of any type of UCD varied between herds from 0 to 62%, and at least one UCD lesion was found in 98 of the 99 included herds (Figure 3).

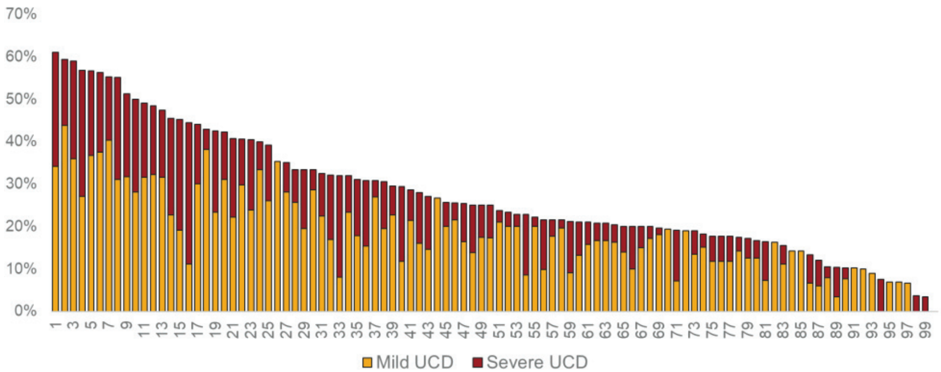

Figure 3. Within-herd proportions (%) of cows affected by mild and severe udder cleft dermatitis (UCD) in 99 Swedish dairy herds visited in 2014 – 2015.

The mean incidence rate of new cases of UCD in paper II was 0.5 cases per cow-year at risk. For mild and severe UCD, the incidence rates were 0.4 and 0.2 cases per cow-year at risk, respectively. The within-herd incidence rates varied between visits but there was no clear seasonal pattern.

The observed duration of UCD was estimated in paper II, but as most cases had an unknown start or end of the UCD case, the duration was most likely underestimated. The median observed duration of UCD was 12 weeks and cases including severe UCD had a longer duration (median 30 weeks) compared to cases with only mild UCD (median 6 weeks).

In paper IV, a spontaneous recovery was seen in 38% of the cows with UCD. The recovery rate for cows with only mild UCD was 59%, and for cases including severe UCD 15%. Most of the cows that recovered from cases of only mild UCD did so within approximately 4 – 8 weeks after their first observation of UCD. For cows with severe UCD, no recovery was seen in cows with more than 26 weeks since the first observation of UCD.

Moreover, 47% of the cows that recovered had a recurrent case of UCD, of which some recovered again.

4.2 Risk factor analyses (I-II, IV)

4.2.1 Cow-related risk factors

An overview of risk factors associated with UCD in papers I – II and factors associated with recovery in paper IV is presented in Table 2.

Factors associated with having UCD (paper I)

Cow-related factors associated with the risk of any type of UCD were breed, fore udder attachment, I/F at the fore udder attachment and parity. These associations were also found when analysing risk factors for mild and severe UCD separately, as were some additional risk factors presented below.

Breed (SR and SR x SH compared to SH cows), fore udder attachment (intermediate compared to strong) and having a hock lesion increased the risk of mild UCD. In addition, an interaction was seen between parity and I/F at the fore udder attachment. For cows with I/F, the risk for mild UCD increased in the first and second parity compared to cows without I/F in the same parity, whereas for cows in the third or higher parity, the risk was not affected by I/F.

For severe UCD, there was an increased risk for cows with increasing DIM, high milk yield, and higher parity. Cows of the SH breed had a lower risk of severe UCD than SR and SH × SR cows. Moreover, an interaction was seen between breed and fore udder attachment. The risk for severe UCD was lower for SH cows with a strong fore udder attachment compared to SH cows with intermediate or loose fore udder attachment, whereas SR cows with a loose compared to an intermediate fore udder attachment had a lower risk of UCD.

Factors associated with transitions to UCD (paper II)

As the risk factors and their importance were the same for transitions to any type of and mild UCD, only the results for mild and severe UCD are presented below.

The risk for a transition to mild UCD increased with increasing DIM. Among cows without an I/F at the fore udder attachment, the risk for a transition to mild UCD was higher for SR cows than for SH cows and other breeds. However, for cows with an I/F, there was no difference between breeds. Within breed, the risk for a transition to mild UCD increased for SH cows and cows of other breeds with I/F compared to cows of the same breed without I/F. In addition, cows with a low milk urea level had a lower risk of a transition to mild UCD.

The risk for a transition to severe UCD increased with higher milk yield, and for cows that had mild UCD at the previous observation. The risk for a transition to severe UCD tended to be lower in second parity cows compared to first parity cows.

Factors associated with recovery from UCD (paper IV)

Cases that included observations of severe UCD had a lower chance of recovery compared to cases of only mild UCD. In the model which only included UCD cases of a known start, a lower chance of recovery was seen in cows with the highest mean daily milk yield (≥ 38 kg) compared to cows that milked 31 – 37 kg, and in third parity cows compared to first parity cows and cows in fourth or higher parity.

In the model that included all UCD cases, higher parity was associated with a lower chance of recovery. For cows with only mild UCD, the chance of recovery was higher by 4 – 8 weeks since the first observation of UCD compared to time periods with more weeks since the first UCD observation.

4.2.2 Herd-related risk factors (paper I)

For any type of UCD, as well as for severe UCD, cows in herds with mattresses had a higher risk for UCD than cows in herds with rubber mats or other cubicle bases. Also, two herd health key performance indicators were associated with any type of UCD (Table 2).

Herd factors associated with increased risk of severe UCD included three housing-related factors and two herd health key performance indicators.

Table 2. *Overview of factors significantly associated with an increased or decreased (indicated by arrows) risk of udder cleft dermatitis (UCD) and chance of recovery from UCD in the final statistical models of papers I – II and IV.*

Factors associated with:	Having UCD			Transitions to UCD			Recovery from UCD
Variable	Any type	Mild	Severe	Any type	Mild	Severe	
Cow-related factors							
Having a hock lesion		↑					
High or intermediate compared to low urea level				↑	↑		
Higher milk yield			↑			↑	↓
Higher Parity	↑	↑	↑				↓
I/F[1] at fore udder attachment	↑	↑		↑	↑		
Lack of strong fore udder attachment	↑	↑	↑				
Long duration of UCD lesion	×[2]	×	×	×	×	×	↓
Mild UCD at the previous observation	×	×	×	×	×	↑	×
More days in milk			↑	↑	↑		
Swedish Red compared to Swedish Holstein	↑	↑	↑	↑	↑		
UCD case including severe UCD	×	×	×	×	×	×	↓
Herd-related factors							
Cubicles installed 2001-2005 compared to before 2001			↑	×	×	×	×
High proportion of not inseminated heifers >17mo				×	×	×	×
High rate of culling due to hoof and leg disease	↑	↑		×	×	×	×
High rate of culling due to udder diseases			↑	×	×	×	×
High rate of culling of 1st parity cows in early lactation			↑	×	×	×	×
High rate of reported VTCM[3]	↑		↑	×	×	×	×
Rubber mattresses in cubicles (vs. rubber mats)	↑		↑	×	×	×	×
Shorter cubicle length			↑	×	×	×	×

[1] I/F = Indentation or fold at fore udder attachment; [2] × = Not investigated; [3] VTCM = Veterinary-treated clinical mastitis.

4.3 Microbiota of UCD lesions in comparison with healthy skin (III)

4.3.1 The overall microbiota

The majority of classified reads within all samples belonged to the Bacteria domain. Archaeal and fungal reads represented on average around 5% of the classified reads, and there was a higher proportion of Archaea in samples from healthy skin (control samples) compared to UCD samples. The proportions of viral and protozoan reads were very low (< 1% in all samples), and were thus not investigated further. In addition, the proportion of *Sarcoptes scabiei* was low in all samples.

4.3.2 Bacterial abundance

The bacterial microbiota differed between control samples and mild and severe UCD samples. The observed differences included both bacterial abundance and diversity. In addition, the PCA showed that control samples clustered together, whereas the UCD samples clustered in subgroups. Based on the PCA and the bacterial abundance in different samples, three subgroups of UCD samples could be identified. The largest subgroup included both mild and severe UCD samples, and were characterized by a large proportion of the phylum Actinobacteria and several *Corynebacterium* spp., in which one or a few species had increased in proportion and represented a high percentage of the total number of classified reads. In a smaller subgroup, mainly including mild UCD samples, *Staphylococcus* spp. had increased in proportion, and in the third subgroup, including severe lesions only, a more anaerobic microbiota was seen, including a relatively high proportion of the genera *Trueperella*, *Fusobacterium* and *Porphyromonas*. In general, the healthy control samples had a higher proportion of the phylum *Proteobacteria* and the genera *Bifidobacterium* and *Lactobacillus* compared to samples from mild and severe UCD lesions.

In addition, the Shannon diversity index showed that the diversity of bacterial genera and species was higher in control samples compared to mild and severe UCD samples. For bacterial species, the diversity was also higher in samples from mild UCD compared to samples from severe lesions.

4.3.3 Abundance of other microorganisms

The proportion of reads classified as Archaea differed between sample types, with a higher abundance in control samples compared to UCD samples, and a higher abundance in mild UCD samples compared to severe UCD samples. The majority of the archaeal reads within control samples belonged to the genus *Methanobrevibacter,* whereas the archaeal reads in samples from mild and severe lesions had a larger diversity, with several other genera present.

Apart from one mild UCD sample with a high proportion of fungal reads, fungi represented only a small proportion of the classified reads within all sample types, and no clear pattern or difference between sample types was observed.

4.4 Effects of UCD on udder health and culling (I-III)

In paper I, we analysed any type of, mild, and severe UCD as explanatory variables for the outcomes milk SCC, VTCM and culling. No associations between UCD and these outcomes were seen when parity was included in the model as a confounder.

In paper II, a recent transition to UCD (any type of, mild, or severe) was investigated as explanatory variables for the outcomes milk SCC and VTCM. A transition to severe UCD increased the odds for VTCM, also when potential confounders were included in the model. No other associations were seen between the explanatory variables and the investigated outcomes.

In paper III, we investigated the pathogens that are considered to be common mastitis-causing bacterial species in Sweden and that represented at least 1% of the reads in at least one sample. Several *Staphylococcus* spp. were found i.e. *S. aureus, S. epidermidis, S. haemolyticus* and *S. simulans*. The proportions of these bacteria were generally low in all sample types, and apart from a higher proportion of *S. aureus* in control samples compared to severe UCD samples, no differences between sample types were seen. In addition, *Trueperella pyogenes* was more frequent in samples from severe lesions compared to samples from the healthy control cows and mild UCD samples.

4.5 Effect of topical treatment of UCD (IV)

There was no significant effect of the treatment, although a weak tendency towards a higher probability of a high UCD score (more severe UCD) was seen in the treatment group than in the control group. A high UCD score on day 1 and a long duration of UCD before the start of the study increased the risk of a high UCD score day on 56. In addition, a strong fore udder attachment, compared to a loose, was associated with a lower UCD score day on 56.

5 General discussion

Within this section, the most important findings in papers I-IV are discussed, as well as some theories about the etiology and underlying causes of the development of UCD and practical implications of the thesis work.

5.1 Prevalence, incidence, duration and recovery (I-II, IV)

The prevalence of UCD in Swedish dairy cows was considerably higher than in a previous Swedish study that used an almost identical scoring system (27% vs 18%; Persson Waller et al., 2014). It was also higher than in a Dutch study (5%), although a different scoring system was used in that study, making comparisons difficult (Riekerink et al., 2014). The difference between the two Swedish studies could be related to an actual increase in UCD prevalence but could also be due to differences in scoring between individual observers or other, unknown, factors. Although UCD lesions were found in almost all herds, in line with previous studies (Persson Waller et al., 2014; Riekerink et al., 2014), there was a large variation in the total within-herd prevalence of UCD indicating that herd factors may affect UCD prevalence.

The incidence rate of UCD was high, with 0.5 new cases per cow-year at risk, which can be compared with the incidence of mastitis (the most common veterinary-treated disease in Swedish dairy cows) which was 0.1 new cases per cow-year at risk in Sweden between September 2018 and August 2019 (Växa Sverige, 2020b). As the incidence of UCD was investigated in a small number of herds with a known medium to high UCD prevalence, inferences to the whole population of Swedish dairy herds are uncertain, although it is likely that, at the very least, herds with such a medium to high prevalence have similar incidence rates. The incidence was lower than in a Dutch study in which the herds were chosen based on a known UCD prevalence of at least 6% (Bouma et al., 2016).

Comparisons with this study might, however, not be appropriate as there were differences in study design and visit intervals, as well as differences in the statistical methods used.

The duration of UCD differed depending on severity of the lesion, with a longer duration for cases including severe UCD, which was in line with the results of Bouma et al. (2016). The overall duration of UCD was, however, probably underestimated due to the limited study period. There was also a large variation between cows; some cows appeared to only be affected by short, mild cases of UCD while other cows had severe UCD for the entire year-long study period.

Less than 40% of the cows with UCD made a spontaneous recovery during the study period, and of those cows, almost 50% had a recurrent case of UCD after the recovery. The chance of recovery was markedly lower if the duration of UCD was long and if the cow had observations of severe UCD. In line with our results, Bouma et al. (2016) also found a higher recovery rate in mild UCD cases compared to severe cases.

The results clearly indicate that UCD is a common problem in Swedish free-stall dairy herds. The high incidence rates, the long duration and low recovery rate as well as the risk for recurrent cases all contribute to the high prevalence of UCD found in several herds. Poor recovery and high risk for recurrence are also characteristic of human chronic wounds with underlying pathology contributing to delayed healing, for example, venous leg ulcers and diabetic foot ulcers (Wu et al., 2019). To reduce the prevalence of UCD in Swedish dairy cows, both preventive measures and treatment strategies are warranted.

5.2 Risk factors for UCD (I-II)

5.2.1 Cow-related risk factors for having UCD and for transitions to UCD

In paper I, breed, udder conformation, and parity were factors associated with having any type of UCD, whereas in paper II, breed, udder conformation and DIM were associated with a transition from unaffected to any type of UCD.

In line with Persson Waller et al. (2014), our results show that SR cows have a higher risk for UCD than SH cows. Also, when investigating risk factors separately for mild or severe UCD, a higher risk for having both mild and severe UCD, as well as an increased risk for a transition from unaffected to mild UCD, were seen for SR compared to SH cows. In several analyses, we found interactions between breed and udder conformation, indicating that the individual udder conformation affects the risk differently depending on breed, which could be related to genetic differences. In brief, traits that have previously been identified as risk factors for UCD, such as the lack of a strong fore udder attachment and I/F at the fore udder attachment, appear to affect the risk primarily in SH cows, whereas these associations are not as clear for SR cows. In general, according to breeding evaluations, SH cows have stronger udder support and less deep udders compared to SR cows (Växa Sverige 2008). The udder support was not investigated in our studies, but it was seen that the udders of SR cows were generally scored as deeper in paper II, compared to the udders of SH cows and other breeds (data not shown). Even if this correlation was not large enough to be deemed as autocorrelation, it could still have affected the statistical models and be a part of the explanation as to why UCD is more common in SR than in SH cows. Deep udders have been shown to increase the risk for UCD in a Danish study (Hansen & Nissen, 2010). If the general udder conformation of SR cows predisposes them to UCD, it is logical that the udder conformation of individual cows is more important for SH cows. If the udder support is related to a stronger suspensory apparatus in SH cows, this could also be related to a lower risk of UCD (discussed below). A hereditary predisposition to UCD cannot be ruled out, and genetic studies of the condition would be of interest.

In our analyses, as well as in previous studies (Persson Waller et al., 2014; Riekerink et al., 2014), the lack of a strong fore udder attachment and I/F at the fore udder attachment increased the risk for having UCD. This affected the risk of having mild and severe UCD (paper I), and also the risk of a transition to mild UCD, whereas no udder conformation trait was associated with a transition to severe UCD (paper II). A lack of a strong fore udder attachment, and particularly an I/F at the fore udder attachment, often results in increased presence of skin folds and subsequent friction and lack of air circulation in the area of UCD, and could thus predispose to an impaired skin barrier. A common clinical manifestation of UCD is the presence of erythema that extends to the ventral anterior parts of the udder (see Figure 1, UCD score 2). This type of erythema is similar to what can be seen in intertrigo, and could be an indication of skin irritation resulting from friction between skin surfaces when the cow lies down, or due to friction between permanent skin folds of the udder.

It is known that udder conformation changes with increased parity (Ral et al., 1988) and increased DIM (Gustafsson, 1998), resulting in looser fore udder attachment and increased udder depth. The increased risk for having severe UCD (paper I) and the increased risk for transitions from unaffected to any type of, and mild, UCD (paper II) with increased DIM are in line with previous studies of having UCD (Hansen & Nissen, 2010; Bouma et al., 2016). This increased risk might be related to such changes in udder conformation. It is also possible that physiological changes occurring in early lactation, such as increased pressure and reduced blood flow in the udder as well as stretching of the skin in early lactation (discussed below in relation to milk yield) could cause skin damage or impaired function of the skin barrier. Although these changes occur in early lactation, it could be speculated that it takes a while for a lesion to develop and that they therefore become more common later in lactation.

In line with several previous studies (Warnick et al., 2002; Persson Waller et al., 2014; Bouma et al., 2016), we found that the risk for having UCD increased with parity. Apart from the associations between parity and udder conformation discussed above, it is also possible that older cows are more susceptible to UCD as they are also more susceptible to other conditions, including mastitis and hock lesions (Nyman et al., 2007; Kester et al., 2014). In addition, the elasticity of the connective tissue in the suspensory apparatus deteriorates with age, resulting in gradual loss of udder support and a subsequent increased udder depth (Blowey & Edmondson, 2010; Persson Waller, 2018). It is possible that the skin at the fore udder attachment is damaged by the tension of the udder skin due to the loss of udder support and thus becomes more likely to develop UCD lesions. The overall elasticity of the skin also deteriorates with age, which could affect the risk of a damaged skin barrier (Blowey & Edmondson, 2010).

In contrast, we found no association between parity and the risk of a transition from unaffected to UCD. The differences between the studies might be related to the long duration of UCD (sometimes overlapping several lactations), leading to an accumulation of cases in older cows, or to other complex associations between parity, DIM, and udder conformation that may affect the development and duration of UCD and that could also have affected the statistical analyses.

A low urea level was associated with a lower risk of transition to any type of, and mild UCD (paper II), a finding that has not been previously reported. Even though diet is known to affect skin conditions in humans (Katta & Kramer, 2018; Bonamonte et al., 2019) and milk urea level is an indicator of the protein balance of the feed, where high urea levels indicates too high protein content (Växa Sverige, 2020b), this finding needs to be further investigated as it also might be a spurious finding and not a true risk factor.

Risk factors associated with having mild UCD, and transitions to mild UCD were similar to those found for any type of UCD. However, having a hock lesion was associated with having mild UCD in paper I. This could be related to common risk factors for having UCD and hock lesions, such as a generally more sensitive skin. In addition, the association between hock lesions and lameness might cause cows with hock lesions to alter their behavior and spend more time lying down (King et al., 2017). Longer lying bouts may expose the udder to dirty bedding material and also increase the risk of an enclosed environment due to skin folds at the fore udder attachment, which could be related to an increased risk for UCD.

Factors only associated with severe UCD

High milk yield was associated with an increased risk of having severe UCD (paper I) and transitions to severe UCD (paper II). This is in line with some previous studies of UCD in which high milk yield in individual cows (Persson Waller et al., 2014) and high herd level milk yield (Persson Waller et al., 2014; Riekerink et al., 2014) were associated with a higher risk of having any type of UCD.

The engorgement of the udder and subsequent skin stretching in early lactation might be more pronounced for high-yielding cows (Lin et al., 1987; Harris et al., 1991), and could possibly impair the skin barrier. In addition, a high milk yield could result in greater pressure within the udder, leading to reduced blood circulation and therefore, reduced oxygen levels and risk of tissue necrosis. Thus, blood flow measurements and histological examinations of the udder skin in early lactation could be of interest for understanding UCD etiology. In paper I, cows milked thrice daily compared to those milked twice a day had a lower risk for severe UCD in the univariable analysis and in the sub-model for management factors, a finding that supports the theory of udder pressure as a contributing factor to UCD. Cows milked three times per day generally have a higher milk yield, but the more frequent milkings might reduce the udder pressure and thus reduce the risk for UCD.

Another result of high milk yield is increased negative energy balance in early lactation. Cows with a higher production level and increased negative energy balance have less resistance to reproductive diseases and laminitis (Collard et al., 2000; Oltenacu & Broom, 2010) and might also be more susceptible to UCD. A negative energy balance, or malnutrition, is associated with impaired wound healing in humans (Barbul & Purtill, 1994; Harris & Fraser, 2004; Stechmiller, 2010). Thus, it could be speculated that high yielding cows have a poorer ability to heal lesions compared to low yielding cows, which might lead to a higher risk of severe UCD. There are complex associations between milk yield, negative energy balance and parity, and they might influence the risk of UCD in combination with each other.

The longitudinal study design made it possible to include mild UCD as a risk factor for transitions to severe UCD, and having mild UCD markedly increased the risk for severe lesions. It was also seen that transitions between mild and severe UCD were common. The finding emphasizes the importance of preventing and curing mild lesions, even though they are harder to detect.

5.2.2 Herd-related risk factors

The large number of herds included in paper I made it possible to investigate herd factors associated with UCD. The herds had a large variation in prevalence of mild and severe UCD prevalence, which indicate that such factors exist. However, specific risk factors that actually affect the risk of UCD are hard to pinpoint. For each herd, management factors, environment and resources differed from other herds, and these differences were difficult to group into comparable categories for the statistical analyses. Even though we found some herd factors affecting the risk of UCD, the statistical models indicated that there were still unidentified factors that affected the risk of UCD.

Some herd health key performance indicators were associated with UCD. These indicators are used as a type of benchmarking and were developed by the former Swedish Dairy Association. It is likely that the associations between these indicators and UCD are related to certain, unidentified, management factors that affect both the herd performance indicators and the UCD prevalence rather than a direct association.

For any type of UCD as well as for severe UCD, cows housed in free-stalls with rubber mattresses had a higher risk of severe UCD than cows housed on rubber mats. This could be due to a warmer and moister environment for the udders of cows lying on mattresses, which are usually thicker and softer than mats. Also, in several herds, the mattress filling had indentations where urine and manure could accumulate in the cubicles, which could be harmful to the skin barrier.

Few studies have investigated herd-related factors affecting UCD and as previously mentioned, such studies can be challenging as all dairy farms are unique, and because complex relationships between the cow and her environment may affect the risk of UCD.

5.3 Factors associated with recovery from UCD (IV)

The longitudinal study design made it possible to investigate factors that affected recovery from UCD. Most cows that recovered had a UCD case of short duration and only mild observations, which is in line with Bouma et al. (2016) who found a higher recovery rate for mild UCD cases compared to severe. The finding that longer duration of UCD, as well as severe lesions compared to mild, reduced the chance of recovery was clear in the statistical models. The overall findings indicate that the chance of recovery is markedly lower if the duration of UCD is around 12 weeks or longer compared to a shorter duration. Very few cows recovered if the duration of UCD was around 18 weeks or longer. The results of the present study are similar to those found in studies on human chronic wounds, such as diabetic foot ulcers and venous leg ulcers, in which duration, wound size and wound severity have been shown to affect time to recovery (Margolis et al., 2002; Vowden et al., 2008).

In addition, higher parity cows had a lower chance of recovery, which is in line with the finding from paper I that the prevalence of UCD is higher in older cows. This finding could be related to a poorer capacity to recover from UCD, possibly due to general skin-related factors in older cows, such as reduced skin elasticity and an impaired wound-healing capacity, as discussed above.

5.4 The role of microorganisms in UCD (III)

Based on the clinical appearance of severe UCD lesions, the finding of bacteria in the lesions is hardly surprising, and bacteria were also the most common finding in all samples in our study. However, the clinical implications of these bacteria are harder to evaluate. As previously mentioned, several infectious agents have been associated with severe UCD, although there is no consensus regarding their role in the development of UCD. To our knowledge, the presence of microorganisms in mild UCD lesions has not been previously investigated.

Several of the bacteria previously identified in severe UCD lesions through unspecific bacteriological culturing (Beattie & Taylor, 2000; Warnick et al., 2002; Persson Waller, 2003) were also identified in our study by shotgun metagenomic sequencing, and by Sorge et al. (2019) by 16S amplicon sequencing. A great advantage of the two latter studies is the inclusion of control samples, comparing the microbiota of UCD lesions with healthy skin from the same body site. In both studies, a decreased diversity was evident in UCD samples compared to controls. Such decreased diversity and increased abundance of certain, potentially pathogenic, species can be defined as dysbiosis of the microbiota, something that is seen in several pathological conditions associated with the human gut and skin, including Crohn's disease, atopic dermatitis and diabetic foot ulcers (Gardiner et al., 2017; Pascal et al., 2017; Rodrigues Hoffmann, 2017).

In several mild and severe UCD samples, the abundance of *Corynebacterium* spp. was higher than that of other species, whereas *Staphylococcus* spp. were seen in high abundance in several mild UCD lesions. Both these bacterial genera are associated with normal skin in animals and humans, and they are also associated with human chronic wounds (Dowd et al., 2008; Johnson et al., 2018). Several severe lesions also displayed a more anaerobic microbiota and the findings were similar to those of Sorge et al. (2019), including *Trueperella pyogenes, Fusobacterium necrophorum* and *Porphyromonas* spp. These bacteria are well-known opportunistic pathogens in other bovine conditions, such as abscesses and interdigital phlegmon. They are also often found together, and can form biofilm in certain environments (Ribeiro et al., 2015; Lockhart et al., 2017).

The fact that the bacteria causing dysbiosis differed between UCD samples indicate that no specific bacteria are essential for the development of UCD, but that opportunistic bacteria may increase in proportion as the local environment changes as a UCD lesion progresses. In the case where we sampled a severe UCD lesion twice, once when it was recently developed, and again after around three months, a shift towards a more anaerobic microbiota was seen. Even though this solitary finding cannot be used to draw any firm conclusions, it could be speculated that the anaerobic microbiota seen in some UCD lesions is an indication of a chronic wound. Also, during a pilot study we performed histopathological examinations of stained tape samples from UCD lesions (data not shown). Samples from severe UCD revealed a large amount of coccoid and rod-shaped bacteria but very few immune cells, and gave the impression of a bacterial colonization rather than an infection (Kerstin Bergvall, personal communication). Tape samples from mild lesions and healthy skin revealed visually lower bacterial density compared to the severe lesion samples.

The involvement of *Treponema* spp. in UCD has been discussed (Boyer & Singleton, 1998; Evans et al., 2010). However, the occasional findings of these bacteria in UCD lesions suggest that they are of no major importance to the development or pathogenesis. This is also in line with our results in paper III, as well as the results of Sorge et al. (2019), in which *Treponema* spp. were found in low numbers in UCD samples. Moreover, in paper I, we found no associations between UCD and claw trimming results or presence of digital dermatitis (reported by the farmer). However, it could be speculated that *Treponema* spp. are opportunistic residents of UCD lesions in herds with a high prevalence of *Treponema* in the environment due to a high prevalence of digital dermatitis.

Mange mites, most commonly *Chorioptes bovis,* but also *Sarcoptes scabiei,* have been subject to investigations in several studies (Warnick et al., 2002; Hansen & Nissen, 2010; Sorge et al., 2019), based on the observation by Allenstein et al. in the early 1990's. None of these studies have, however, found any convincing evidence of mange mites as a cause of UCD, even though mites or mite eggs are occasionally found in the lesions. In paper I, we used farmer interviews to identify problems with ectoparasites in the herds, but found no association between their responses and the risk of UCD. In paper III, we compared the sequenced reads from UCD lesions and healthy skin samples with the genome for *Sarcoptes scabiei* (not present in Sweden) and found less than 1% matching sequences in all samples. Unfortunately, it was not possible to investigate the presence of *Chorioptes bovis* as no genome sequence was available in the NCBI database. Despite this, it seems unlikely that mange mites are important to the etiology of UCD, as concluded by several other authors (Warnick et al., 2002; Hansen & Nissen, 2010; Sorge et al., 2019).

In conclusion, our results indicate that the microbiota of UCD lesions is similar to what can be found in similar conditions in other species, such as atopic dermatitis (Kong et al., 2012; Bradley et al., 2016) and human chronic wounds (Dowd et al., 2008; Gardiner et al., 2017), and that no primary pathogen is involved in the etiology. Previous microbiological studies of UCD have, to our knowledge, mainly included what we would define as severe lesions. Our results show that the microbiota of most mild UCD lesions also display signs of dysbiosis and that the mild UCD samples were more similar to severe UCD samples than to control samples from healthy skin.

5.5 Possible consequences of UCD (I-III)

5.5.1 Associations between UCD and mastitis

The possible association between UCD and an increased risk for mastitis was investigated epidemiologically in papers I and II, and microbiologically in paper III.

In paper I, we found no association between UCD and VTCM when parity was accounted for in the statistical models, which was in contrast to the results of a previous Swedish UCD study (Persson Waller et al., 2014). However, we know that diseases are underreported (Wolff et al., 2012), which means that the retrieved data might not be complete. Associations between UCD and milk SCC were not found, which is in line with previous studies (Persson Waller et al., 2014; Riekerink et al., 2014). Interestingly, in paper I, some of the investigated herd health key performance indicators related to udder health were associated with severe UCD. These associations were analyzed to investigate risk factors for UCD, but they could also be related to UCD affecting the udder health in the herd. However, they may also be related to other management factors affecting both UCD prevalence and herd udder health.

In paper II, a higher risk for VTCM was seen for cows with a transition to severe UCD which supports the findings of Persson Waller et al. (2014). During this study, VTCM cases were registered by the farmer to avoid the problem of underreporting. It is not known why UCD would increase the risk for VTCM and these findings could be related to common risk factors for UCD and mastitis. It could also be speculated that UCD lesions may act as a reservoir for mastitis-causing pathogens and thus increase the spread of such bacteria in the herd.

To investigate whether UCD lesions may act as such a reservoir, bacteria considered as common mastitis-causing pathogens in Sweden were investigated in paper III. However, these bacteria were only a very small proportion of the microbiota of UCD lesions, indicating that UCD does not function as such a reservoir. The proportion of *Staphylococcus aureus* was, for example, higher in healthy skin samples than in severe UCD samples. An exception was the presence of *Trueperella pyogenes* in severe UCD lesions, a bacterium known to cause mastitis in heifers and dry cows during the summer (Ribeiro et al., 2015). Considering the opportunistic nature of *T. pyogenes*, its presence in severe UCD lesions is not surprising and this probably has little consequence for the risk of mastitis in affected cows. However, during the summer, severe UCD lesions may increase the risk of flies spreading *T. pyogenes* and thus increase the specific risk of so called “summer mastitis”.

5.5.2 Other potential consequences of UCD

Associations between UCD and culling have, to our knowledge, not been previously investigated and our results suggest that factors other than UCD are more important to the risk of culling. It has also been discussed if UCD could have a negative effect on milk production, but in contrast, our results indicate that high-yielding cows have a higher prevalence of UCD.

5.6 Treatment of UCD (IV)

A treatment strategy for UCD lesions, particularly severe lesions, is warranted by Swedish farmers *and* veterinarians, something that has become even more evident in this project during conversations at herd visits and in other contexts. A pilot study – although not a controlled trial – of the topical spray used in the treatment study reported positive effect on recovery from UCD (Lammers et al., 2017). However, based on our results, the spray containing chelated copper and zinc cannot be recommended for the treatment of UCD lesions as no positive effect on the recovery was found. Similar results were found in a Norwegian study using the same product (Eikeland & Paulsen, 2018). The reasons for the lack of effect are not known. It might be speculated that the antibacterial effect of the copper could be more beneficial for UCD lesions in which certain bacteria, such as *Treponema* spp. are present, and that such bacteria were of little importance to the UCD lesions in our study.

As the origin of UCD lesions most likely is multifactorial rather than infectious, it is possible that the primary focus of treatment strategies should be on creating an optimal environment for healing rather than on the use of antibacterial products. Based on research on human wound care, important features in the treatment of chronic wounds are careful cleaning and debridement, applying products that keep the wound bed moist, as well as adapting the treatment to the individual wound and its characteristics (e.g. facilitate epithelialization, absorb wound secretions and inhibit detrimental bacteria if wound infection is present) (Vowden et al., 2008; Lindholm, 2012). In line with this, van Werven et al. (2018) found that an enzyme alginogel, used in the treatment of human pressure ulcers, had a good effect on severe UCD lesions.

Interestingly, we found that control group cows with severe UCD lesions had a higher improvement rate than cows included in the study on spontaneous recovery. This implies that the thorough cleaning of lesions on day 1 of the treatment study worked as a form of debridement and facilitated healing. However, such conclusions require further investigations. Another important feature of human wound care is to start treatment as early as possible, as both increased duration and wound size are factors that affect the recovery rate (Margolis et al., 2002; Lindholm, 2012). As the duration of UCD lesions reduced the recovery rate, treating UCD lesions at an early stage would probably be beneficial – although we still lack an efficient product for treatment that can be used in Sweden.

5.7 Methodological considerations

5.7.1 Herd visits and data collection

The fact that the same person performed all herd visits and scoring of UCD lesions, hygiene and udder conformation is a strength throughout the project. Nevertheless, the subjective scoring system might have led to intra-observer differences between herds and different visit periods, particularly in cases in which the UCD lesion or the udder shape was "in between" two categories. Most UCD lesions were photographed during herd visits, and these photographs were used to confirm or adjust UCD scores in cases where the score was uncertain.

5.7.2 Microbiological sampling and laboratory methods

When choosing methods for paper III, we performed a pilot study, in which a small number of cows, with and without UCD, were sampled, and the samples were subjected to bacteriological culturing and DNA extraction. We found that commonly used cotton swabs resulted in very low amounts of DNA when extraction was performed without any prior amplification. We therefore decided to use sponges for sampling. In addition, conventional aerobic and anaerobic culturing of the samples proved to be time-consuming and added little to the results of the study, as we know that certain bacteria thrive under such culturing conditions, whereas others do not. Previous microbiological studies of UCD have been performed using swab samples or biopsies. A biopsy could yield more information on both the histopathology of UCD lesions and the microorganisms that are present below the skin surface. For practical reasons, taking biopsies was not possible in this project.

The centrifugation and removal of supernatants after stomachering of the swab samples were performed to concentrate the microbial DNA before extraction, but may have led to the removal of small particles, such as viruses, prior to the extraction. The reason for the high degree of fragmented DNA in some samples is not known. This resulted in a reduced number of samples available for sequencing, and thus, we chose samples in order to achieve an even distribution from different herds and sample types. However, we did not match samples from cows with UCD and control cows according to breed and parity, which was our original intention. A higher number of sequenced samples would have been desirable for the power of the study, but we believe that the achieved results improved the understanding of UCD lesions.

5.7.3 Statistical methods

When performing multiple statistical analyses the risk of making type I errors, i.e. the identification of associations that are not true, increases. Thus, the use of univariable analyses as a screening tool for variables to include in the multivariable analyses (which was performed in papers I, II and IV) might induce such errors. However, when examining complex relationships, it is not always clear which variables to test, and combining univariable and multivariable logistic regression models could reduce the risk of such errors occurring. Even so, the results should always be interpreted in the light of their biological plausibility and preferably also investigated and reinforced in further studies.

In the longitudinal study in particular, the statistical analyses proved challenging. As we wanted to use as much of the collected data as possible, we also included cows with missing observations. For the investigations of factors that influenced the incidence of and the recovery from UCD, we chose between using survival analysis and a transition model, in which the survival analysis would answer the question “how do factors influence the time to an event?” and a transition model would answer the question “what factors influence the possibility of an event?". For paper II, we believed that the factors influencing the development of a UCD lesion was most of interest, and thus used the transition model. However, once a cow has developed a lesion, the factors that affect the time to recovery appeared to be a more appropriate question, particularly for cases in which we knew when a UCD lesion had developed. Thus, survival analysis was used in paper IV.

5.8 Theories of a multifactorial etiology

As discussed in the sections above, a multifactorial origin of UCD is likely. Figure 4 attempts to describe some of the possible causal relationships between the risk factors identified throughout the project and potential underlying causes involved in the development of UCD lesions and that may also affect the severity of UCD.

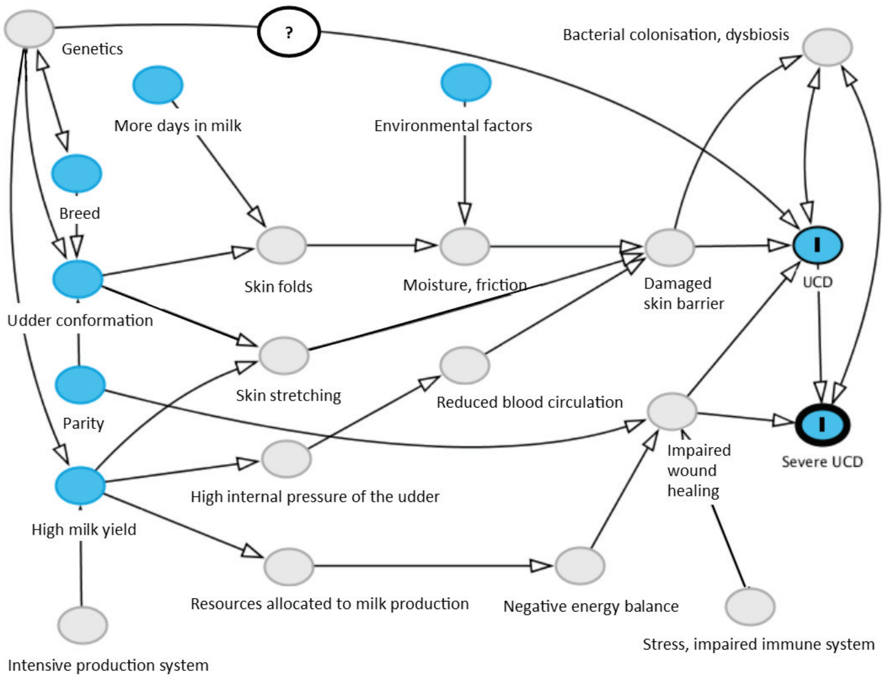

Figure 4. Theories of causal relationships of potential underlying causes (grey circles) for udder cleft dermatitis (UCD) and identified risk factors in epidemiological studies of UCD (blue circles).

5.9 Practical implications

The studies included in this thesis have identified several risk factors for UCD, but found no evidence to support an infectious cause of the lesions. The findings can be used to improve recommendations on prevention and treatment of UCD.

The origin of UCD is most likely multifactorial, and we know that cows with a certain udder conformation have a higher risk of developing lesions. Also, our results, in addition to previous studies on UCD and human chronic wounds, indicate that a lack of air circulation and increased moisture due to skin folds and/or environmental factors could impair the skin barrier, thereby predisposing to UCD. Thus, a dry, clean environment in the cubicles could possibly reduce the risk for UCD.

The localization of the lesions makes them difficult to detect, and the farmer usually becomes aware of lesions when they start to smell, or when exudations of blood or pus contaminate milking equipment or the floor, and such severe lesions are known to have a long duration. According to human chronic wound care, early treatment of lesions is important for improving the chance of recovery. In herds that experience problems with UCD, such as a high prevalence and a high proportion of severe lesions, a more proactive identification of mild and recently developed lesions might result in better treatment results. Even though no products on the Swedish market have proven effective against UCD, based on the findings of this thesis a potential treatment strategy is suggested below.

5.9.1 Treatment recommendations for UCD

These recommendations are based on a combination of current recommendations for human chronic wound care (i.e. Lindholm, 2012), the findings in this thesis and a previous study on UCD treatment (van Werven et al., 2018). The recommendations may change due to future research results, and as more products become available.

- Thorough cleaning and debridement of UCD lesions, e.g. with clean udder cloths soaked or moistened in clean warm water. It is important to remove as much as possible of crusts, necrotic tissue and fibrin (Figure 5). Such cleaning should, however, not be repeated regularly, as repeated cleaning could disturb the healing process of the wound.
- Application of topical treatment. The effect of different products may depend on if the wound is exudative or dry, or if it shows signs of delayed healing and/or secondary infection. An enzyme alginogel had a good effect on severe UCD lesions in a Dutch treatment study, but no such product is currently

available in Sweden (although such a product could hopefully be approved for use in the future). Medical honey is a product with similar properties, with proven anti-inflammatory and antibacterial effects, as well as the ability to keep the wound moist. For mild lesions located within skin folds, a skin protective cream, such as zinc paste could be useful. Both medical honey and zinc are effective treatments for intertrigo in humans (Nijhuis et al., 2012), and could also be an alternative for treating UCD, although they have not been tested on such lesions. A condition for any topical treatment is that the product must be approved for use in milk-producing animals.

- A bad smell of the wound may indicate colonization or secondary infection with anaerobic bacteria, and in such cases a product with antibacterial effect, such as iodine or copper, might be effective. Even though we saw no general effect of the chelated copper and zinc spray in our treatment study, a few cows in the treatment group did improve from a large severe (score 5) lesion to mild or no UCD during the study period, something that was rarely seen in the study of spontaneous recovery. One farmer also reported that severe lesions in treated cows improved after the end of the study. Thus, for such severe lesions, it might be worth testing a similar treatment.
- General recommendations may also include keeping the cow in a dry and clean environment. Moreover, as stress and malnutrition have been shown to impair healing of human wounds (Walburn et al., 2009; Stechmiller, 2010), factors that improve the overall health of the cow, such as reduced stress, a balanced feeding plan and freedom from other diseases may also improve the recovery rates of UCD lesions.

Figure 5. Illustration of two severe UCD lesions before (A) and after (B) cleaning/debridement with clean udder cloths soaked in warm water.

6 Conclusions

The studies included in this thesis have contributed to an increased understanding of the epidemiology and etiology of UCD. Although the increased understanding can be used to improve recommendations on how to prevent and treat UCD lesions, further studies on UCD are warranted.

Specific conclusions generated are:

- UCD is common among Swedish dairy cows, although most lesions are mild. The prevalence of mild and severe UCD differs between herds.
- Several important cow-level risk factors for having UCD were identified, such as udder conformation traits and breed. Some risk factors affected the risk of both mild and severe UCD, while others were specific to either mild or severe lesions. Some herd-related risk factors were also identified, but they explained only a small part of the between-herd variation.
- The incidence of UCD was high in the investigated herds, and risk factors for transitions to UCD were similar to those for having UCD. Severe UCD often had long duration, whereas cows with mild lesions and short duration had the highest chance of spontaneous recovery. Recurrent cases of UCD were common.
- The microbiota of mild and severe UCD lesions displayed a dysbiosis, with decreased diversity and a high abundance of certain bacterial species compared to the microbiota of udder skin from healthy cows. However, no specific pathogens were associated with UCD, which supports the theory of a multifactorial origin of the lesions.
- Severe UCD was associated with an increased risk for clinical mastitis, but common mastitis-causing bacteria were a rare finding in the UCD microbiota. UCD did not affect the risk of culling.
- The tested topical copper and zinc spray cannot be recommended for the treatment of UCD lesions in general.

7 Future perspectives

Even though the knowledge on UCD has increased, there are questions regarding these lesions that remain, and there are new questions that have been raised. Some aspects of UCD that warrants further investigation are listed below.

Could predisposition for UCD be related to genetic traits?

As previously mentioned, the increased risk for UCD in SR cows compared to SH cows could be related to differences in udder conformation. Such differences, or other genetic traits associated with UCD could possibly be identified through genetic studies of UCD. In addition, as the cows within a herd are generally related to each other, such genetic traits could also explain the variation in UCD prevalence between herds. If genetic traits associated with UCD could be identified, there is a possibility that the condition could be prevented through implementing such traits in breeding programs.

Are there other herd factors that could affect the UCD prevalence?

There are clear indications that herd factors affect the risk of UCD, however, we could only find a few such factors, and they only explained a small part of the between-herd variation. Apart from genetic studies, further epidemiological studies investigating herd factors would be of interest. In such studies, a more defined research question in relation to UCD would probably be more appropriate, to avoid investigating too many factors at once. Related to the findings within this thesis, one such factor could be production intensity: Is the risk for UCD higher in dairy herds focused on intensive production e.g. high production, high amount of concentrated feedstuff, high stocking density and large herd size?

What should we use to treat UCD lesions?

As the topical spray in our treatment study did not improve the recovery rate of UCD lesions during the study period, further treatment studies are warranted! Our overall results indicate that the origin of UCD is multifactorial, and that in similarity to human chronic wounds, treatment should be focused on creating an optimal local environment to stimulate wound healing. This may include cleaning and debridement of UCD lesions, as well as topical application of suitable products. However, the challenge is to find suitable products that are approved for treatment of bovine wounds and available on the Swedish market. Medical honey or zinc paste might be candidates for future treatment studies on UCD, if approved for use in dairy cows according to legislation and food safety.

Can the development of UCD be related to specific physiological changes at the fore udder attachment?

Detailed studies on the pathogenesis of UCD would be of interest to increase the understanding of the condition. Such studies could, for example, include histopathological and microbiological investigations, as well as measurements of blood flow and internal pressure in the area of the fore udder attachment.

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Popular science summary

Udder cleft dermatitis (UCD), or udder sores, is a skin condition that develops at the fore udder attachment of dairy cows, where the udder attaches to the abdominal wall, or between the udder halves. The UCD lesions vary in appearance from mild, eczematous skin changes, to severe, with wounds that often become large, foul smelling and purulent. The lesions impair the welfare of the affected cows and may also become a hygiene issue during milking and in the stable. In addition, a previous Swedish study found associations between UCD and mastitis (inflammation of the udder). It is not clear why UCD lesions develop, but some risk factors are known. The aim of this thesis was to increase the understanding of the prevalence of UCD in Swedish dairy cattle herds, and to analyse potential risk factors for UCD. We also investigated which microorganisms are present in the lesions and if there were associations between UCD and mastitis, and we studied spontaneous recovery and performed a small treatment study of UCD.

The first part of the project involved 99 dairy cattle herds in the southern and central parts of Sweden. All herds had free-stall housing, milked in milking parlors and were of average Swedish herd size. Because the cows were milked in parlors, it was possible to examine them for UCD during milking. Thus, herd visits were thus performed during milking. Also, in addition to scoring UCD, hygiene and the udder shape of each cow were evaluated, and the farmer or staff were asked questions about management routines and other herd factors.

In the second part of the project, seven dairy herds were followed for one year. During the study period, all milking cows were examined during herd visits at six-week intervals, in total nine visits per herd. In this study we wanted to investigate factors that affect the development of, and the recovery from, UCD. We also took samples from recently developed UCD lesions to investigate the microorganisms present in the lesions and compare them with samples from healthy skin at the fore udder attachment from cows without UCD. A treatment study was also conducted in four of the herds, in which a spray containing copper and zinc was tested as a topical treatment against UCD.

The results showed that UCD is common in Swedish dairy cows, as almost one third of the more than 3,000 cows that were examined had mild or severe UCD. Around two thirds of the lesions were mild and one third severe. We also found that the lesions often had a long duration, particularly severe UCD, and that the chance of recovery diminished the longer the cow had the UCD lesion. Recurrent cases of UCD was also common.

A higher risk for both having and developing UCD was seen in cows of the Swedish Red breed, compared to Swedish Holstein cows. Cows with a certain udder shape also had higher risk of UCD, mainly cows that lacked a strong fore udder attachment and cows with an indentation or fold at this attachment. UCD was also more common in older cows (older cows did not, however, have a higher risk of developing UCD lesions) and older cows also had less chance of recovering from UCD. Cows with high milk production had a higher risk of both having and developing severe UCD lesions, and we also found that mild lesions often became severe. The microbiological investigations revealed that both mild and severe UCD lesions had a deviating microbiota (the compilation of microorganisms in a certain environment) compared to healthy skin. On healthy skin, a high number of different bacterial species was found, a high diversity, whereas the UCD lesions more often had a high proportion of a single type of bacteria. However, it was not the same type of bacteria that were found in all UCD lesions, and we found no evidence that UCD is caused by a specific microorganism. An association between severe UCD and mastitis was found, but it is not known how UCD affects the risk for mastitis. The topical treatment that was tested had no positive effect on the recovery from UCD.

In conclusion, our studies show that UCD is common in Swedish dairy cows, and that the high prevalence is a combination of a high number of cows developing UCD, particularly in some herds, and that the lesions often have a long duration as well as a high risk of recurrence. Cows with severe UCD have a low chance of recovery and no known effective treatment is currently available in Sweden. The results imply that UCD has underlying causes, such as a certain udder shape, but that no specific microorganism is responsible for the lesions. Further studies on UCD are warranted in order to reveal more about the underlying causes of UCD and identify effective treatment strategies.

Populärvetenskaplig sammanfattning

Juversår är en typ av hudförändringar som uppstår vid den främre juveranfästningen hos mjölkkor, där juvret fäster in i bukväggen, eller mellan juverhalvorna. Juversåren kan variera i utseende från lindriga, eksemliknande förändringar till kraftiga, med sår som ofta blir stora, illaluktande och variga. De medför nedsatt djurvälfärd för drabbade kor, och innebär också risk för försämrad hygien vid mjölkning och i ladugården. En tidigare svensk studie har också hittat samband mellan juversår och mastit (juverinflammation). Det är okänt varför såren uppstår, även om vissa riskfaktorer är kända. Målet med denna avhandling var att öka förståelsen för förekomsten av juversår i svenska mjölkkobesättningar, samt att analysera möjliga riskfaktorer för juversår. Vi har också undersökt vilka mikroorganismer som finns i juversåren och om det finns kopplingar mellan juversår och mastit, samt studerat spontan avläkning och utfört en mindre behandlingsstudie av juversår.

I den första delen av projektet ingick 99 mjölkkobesättningar runtom i södra och mellersta Sverige. Alla besättningar hade lösdrift och mjölkade i mjölkgrop och var medelstora enligt svenska mått. Att korna mjölkades i mjölkgrop gjorde det möjligt att undersöka förekomsten av juversår på ett smidigt sätt under tiden som korna mjölkades. Besättningsbesök gjordes därför vid en mjölkning, och utöver undersökning av förekomsten av juversår bedömdes kornas hygien och juverform, och lantbrukaren eller personal fick också svara på frågor gällande skötselrutiner, stallinredning och andra besättningsfaktorer.

I den andra delen av projektet följdes sju mjölkkobesättningar under ett års tid. Under året undersöktes alla mjölkande kor i besättningen vid besök var sjätte vecka, totalt nio besök per besättning. I denna studie ville vi undersöka faktorer som påverkar uppkomsten och avläkning av juversår, och vi tog också prover från juversår som nyligen utvecklats för att titta på vilka mikroorganismer som förekommer i såren för att sedan jämföra dessa med mikroorganismer på frisk hud vid den främre juveranfästningen hos kor utan juversår. En mindre behandlingsstudie utfördes också i fyra av besättningarna, där en spray innehållande koppar och zink testades som lokalbehandling mot juversår.

Resultaten av studierna visade att juversår är vanligt förekommande hos svenska mjölkkor, då nästan en tredjedel av de över 3 000 undersökta korna hade någon form av juversår. Av såren var cirka två tredjedelar lindriga, och en tredjedel kraftiga. Såren riskerar också att bli mycket långvariga, framför allt de kraftiga, och chansen för avläkning minskar ju längre kon haft sitt juversår. Dessutom såg vi att många kor fick upprepade sår under studieperioden.

En högre risk för att både ha och få juversår sågs för rasen svensk röd och vit boskap jämfört med svensk holstein. Kor med en viss juverform hade också ökad risk, och då framför allt kor som saknade en stark främre juveranfästning och som hade en grop eller ett hudveck vid denna anfästning. Även kons ålder påverkade, det var vanligare med juversår hos äldre kor (dessa hade dock inte större risk för att utveckla nya sår), och de hade lägre chans för avläkning av såren. Kor som producerade mycket mjölk hade högre risk för att både ha och få ett kraftigt juversår jämfört med kor som producerade mindre mjölk, och vi såg också att lindriga sår ofta övergick till kraftiga. De mikrobiologiska undersökningarna visade en störd balans av mikrobiotan (sammansättningen av mikroorganismer i ett visst område) i både kraftiga och lindriga juversår jämfört med mikrobiotan på frisk hud. På frisk hud fanns många olika bakteriearter i små mängder, en stor diversitet, medan juversåren ofta hade större andel av en enstaka bakterieart. Dock var det inte samma arter av bakterier som hittades i alla juversår, och vi fann inga bevis för att juversåren orsakas av en viss bakterie eller annan mikroorganism. Ett samband mellan kraftiga juversår och mastit hittades, men det är inte känt hur juversår påverkar risken för mastit. Den lokalbehandling som testades i den sista studien hade inte någon positiv effekt på avläkningen av juversår.

Sammanfattningsvis fann vi att juversår är vanligt hos svenska mjölkkor, och att den höga förekomsten är en kombination av att många kor får juversår, framför allt i vissa besättningar, samt att såren ofta blir långvariga och att det är stor risk för återkommande fall. Framför allt kraftiga sår har låg chans att läka av, och det finns ingen känd effektiv behandling mot juversår tillgänglig i Sverige idag. Resultaten tyder på att juversår har underliggande orsaker, till exempel en viss juverform, men att det inte är någon speciell mikroorganism som är orsaken. Det behövs dock fler studier om juversår för att ta reda på mer om underliggande orsaker till juversår och för att hitta en effektiv behandlingsstrategi.

Acknowledgements

The work of this thesis was carried out at the Department of Clinical Sciences at the Swedish University of Agricultural Sciences (SLU) and at the Department of Animal Health and Antimicrobial Strategies at the National Veterinary Institute (SVA), Uppsala, Sweden. The project was generously funded by the **Swedish Research Council Formas** (grant number 221–2013–269) and **Stiftelsen Lantbruksforskning** (the Swedish farmers' foundation for agricultural research). Travel grants for presentations at international conferences were kindly provided by **Knut och Alice Wallenbergs Stiftelse (SLU)** and the **Royal Swedish Academy of Agriculture and Forestry (KSLA)**.

There are so many people (and cows) who made this thesis what it is, and I would like to thank each and everyone of you, with specific mentioning of a few:

First and foremost: My main supervisor, **Karin Persson Waller**. Thank you for all your time, encouragement, good advices and never-ending commitment throughout these six years. To me, you are an inspiration and a role model, both in the scientific world, as well as in the world "outside", and I am so happy that you gave me the opportunity to be a part of this project.

Ann Nyman, co-supervisor. Thank you for great statistical guidance and support. Without you, I would probably still be sitting at the computer, cursing at statistical models. Also, thank you for being a genuinely warm and kind person, I am looking forward to being colleagues again :)

Bo Segerman, co-supervisor. Thank you for invaluable help and guidance in the world of metagenomics. One of the disadvantages of leaving SVA will be not being able to beat you at "Hitta ut".

Elisabeth Bagge, co-supervisor. Thank you for sharing your knowledge on bacteria and for always being helpful. And thank you for providing our new garden with orpines.

Ulf Magnusson, co-supervisor. Thank you for valuable advice, for raising important questions and for contributing with a bit of realism when the rest of the group tended to be a bit optimistic.

Former and present Director General of the National Veterinary Institute, **Jens Mattsson** and **Ann Lindberg**. Thank you for letting me perform this work at SVA, and for contributing to an inspiring place to work.

Former and present Head of Department at Clinical Sciences, SLU, **Björn Ekesten** and **Nils Fall,** thank you for letting me be a part of KV and the University.

Märit Pringle, thank you for good advice on bacterial culturing (and non-culturing) as well as plant culturing, gardening and snail euthanasia.

Håkan Landin, thank you for sharing your vast experience and knowledge of dairy cows with me. And for being good company for early morning jogs.

The 101 farmers and over 4,000 cows that participated in the studies. Thank you for your hospitality and kindness throughout this project. For patiently answering questions, for offering coffee and sometimes breakfast after early morning milkings and for sharing a small part of your everyday life with me. And a special thank you to the seven herds and their staff that endured my repeated visits for over one years time, thank you so much for your time spent on this project!

Ylva Sjunnesson, thank you for being a superb coordinator of the PhD education, you have made the administration of the project a thousand times easier.

Ulf Emanuelson, thank you for always being supportive and for contributing with statistical advise. Also, thank you for great supervision for bachelor's as well as master's degree in veterinary medicine, and for finding me a job after the dissertation.

Ian Dohoo, thank you for great statistical advice and for positive encouragement.

All present and former co-workers at DOA and SVA with special mentioning of

Per Wallgren, thank you for your leadership during my time at DOA, always supporting and caring but believing in the freedom and responsibility of the individual. And thank you for all your shared wisdom during these years, I promise to never (again) deliver bad news on a Friday afternoon. Especially not by e-mail.

Maria ”Maja” Persson, thank you so much for all help throughout these years, both in the laboratory and outside. And thanks for always being supportive and positive, fika-time at SVA is not the same without you.

Everyone at **DOA-lab**, for always being helpful, as well as all present and former co-workers at **Lantbrukets djur**, and the rest of **DOA**, thank you for all the coffee breaks and social activities during these years. An extra thanks to **Carina** and **Sara** for helping with administration, and to **Ylva Persson** for excellent general life-coaching.

Karin Ullman and **Harri Ahola**, thank you for useful advice on DNA-extractions, and thank you **Ylva Lindgren**, for helping with samples that I never got around to taking :)

Kerstin Bergvall and **Inger Lilliehöök**, thank you for helping with the methodology and interpreting tape samples.

Former and present **SVA-PhD:s**, thank you for fikas, and shared experiences. And especially I would like to thank **Åsa** and **Anna**, for taking very good care of me when I was new at work, and **Emelie** and **Dinah** for all the Friskis-workouts, chats about important (and unimportant) things and general support.

Former and present “**other” PhD:s**, at SLU, including the valuable members of “**Statistikhjälpen**” and **skrivgruppen**, thank you for good discussions and helpful support, with special mentioning of a few. **Daniel**, for good work in the PhD council, **Emeli**, for being an inspiring lott-odlare, **Niclas** and **Lena** for being great company in Husdjurssektionen, and also great company in general, and **Josef**, for talks about life and microbiotas.

Dorota, thank you for sacrificing a weekend and a full nights sleep when coming with me to Arboga to sample cows in the very early morning, and for being fantastic company during that time, as well as at all other times!

Ida, thank you for being a fantastic, helpful, honest, supportive, fun and generous friend. I am lucky to have you in my life.

Alla vänner och familj utanför SLU och SVA som jag heller inte klarat mig utan, **Moa**, **Signe**, **Monika** och **Karin**, tack för att ni alltid finns där (även om där är lite väl långt bort ibland), tack för alla djupa samtal om livet, alla middagar, sånger och skratt, alla möhippor och fester, och för att ni är ni!

Mamma, **Pappa**, **Tina**, **Sara**, **Camilla** och **Sofia**. Tack för att jag fått växa upp mer er, för er villkorslösa kärlek och för att ni fått mig att tro att jag kan klara allt jag vill.

Johan, som jag inte klarat mig utan under dessa år, och som jag aldrig vill behöva klara mig utan. Tack för att du finns och för att vi är vi.
Sigge och **Billy**. Tack för att ni gör mitt liv till vad det är, och för att ni får mig att inse vad som verkligen är viktigt.

Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2020:23

This thesis investigates the epidemiology and etiology of udder cleft dermatitis (UCD) in Swedish dairy cows. The results show that UCD is common in Swedish dairy herds, and several risk factors for the condition were identified. The duration of UCD is often long, especially if the cow has severe UCD. No specific pathogen was associated with the development of UCD, and the origin of the lesions are most likely multifactorial. An association between severe UCD lesions and mastitis was found.

Lisa Ekman received her postgraduate education at the Department of Clinical Sciences, Swedish University of Agricultural Science, Uppsala. She obtained her degree in veterinary medicine in 2014 at the Faculty of Veterinary Medicine and Animal Science at the same university.

Acta Universitatis Agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

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Online publication of thesis summary: http://pub.epsilon.slu.se/

ISSN 1652-6880
ISBN (print version) 978-91-7760-564-5
ISBN (electronic version) 978-91-7760-565-2