DAIRY CATTLE TRANSITION PERIOD: DISEASE PREVALENCE AND RISK FACTORS IN GRAZING AND FREESTALL SYSTEMS

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Applied Animal Biology)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

July 2019

The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, the dissertation entitled:

DAIRY CATTLE TRANSITION PERIOD: DISEASE PREVALENCE AND RISK FACTORS IN GRAZING AND FREESTALL SYSTEMS

submitted by Rolnei Ruã Darós in partial fulfillment of the requirements for

the degree of Doctor of Philosophy

in Applied Animal Biology

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Abstract

Many dairy cows become ill in the weeks after calving. The goal of this thesis was to study the epidemiology of these 'transition cow' diseases in pasture and confinement systems by undertaking two large observational studies, one in Santa Catarina, Brazil and another in British Columbia, Canada. There is a dearth of research on the prevalence and incidence of transition period diseases in grazing systems. Using a cross sectional approach we measured the prevalence and risk factors for common transition period diseases in 53 small-scale, year-round, grazing dairy herds in Santa Catarina, Brazil. We found that the prevalence of metabolic and infectious diseases in these herds were comparable to those described on high producing indoor systems. Our findings also identified risk factors associated with transition period diseases in these grazing herds. One highly prevalent challenge in indoor systems is lameness, a malady that is often an overlooked in studies of transition period disease. Through a longitudinal study, we followed 455 dairy cows housed indoors on farms located in the lower Fraser Valley region of British Columbia, Canada, to: 1) measure lameness during the prepartum period and 2) assess how lameness contributes to the development of transition diseases. There was a high incidence of lameness during the non-lactating 'dry' period, and cows that were lame during the dry period were more likely to develop transition period diseases. One possible mechanism for this association is via reduced feeding time, as lame cows spent less time feeding than sound cows. Reduced body condition score during the dry period was also associated with increased risk of transition period diseases, independently of lameness and feeding time. I conclude that preventing lameness and body condition loss during the dry period may improve transition health.

Lay Summary

Over 50% of dairy cows become sick after giving birth. Diseases developing shortly after calving are called transition period diseases. The prevalence of these diseases are reasonably well described in confined dairy cattle, yet few studies have explored transition period diseases in grazing dairy cows. In this thesis, I describe the prevalence and risk factors for common transition period diseases in 53 grazing herds in Southern Brazil. Lameness is considered to be one of the most common problems in confined dairy cattle; however, little is known about the relationship between transition period diseases and lameness in indoor housed cows. My research has shown that there is high incidence of lameness during the weeks before calving, and that lame cows are more likely to develop transition period diseases. Throughout this thesis I discuss how changes in management practices may improve cow health in both pasture and confined systems.

Preface

All studies described in the chapters of this thesis were carried accordingly the to the animal care protocols approved by the Universidade Federal de Santa Catarina (Chapter 2, Protocol # PP1237779, 2015 and Protocol # PP00949, 2014) and University of British Columbia (Chapter 3 and 4, Protocol # A15-0084).

A version of Chapter 2 has been published: Daros, RR; Hötzel, MJ; Bran, JA; LeBlanc, SJ and von Keyserlingk, MAG. 2017. Prevalence and risk factors for transition period diseases in grazing dairy cows in Brazil. Prev. Vet. Med. 145: 16 – 22. The paper was co-authored by my supervisor MAGvK and collaborators from the Universidade Federal de Santa Catarina (JAB and MJH) and University of Guelph (SJL). Main concepts, ideas and experimental design were developed by me, MAGvK, MJH and SJL. Data were collected by JAB, 3 research assistants and myself. All co-authors helped interpreting the results and edited drafts.

A version of Chapter 3 has been submitted for publication: Daros, RR; Eriksson, HK; Weary, DM and von Keyserlingk, MAG. Lameness during the dry period: epidemiology and associated factors. The paper was co-authored by my supervisor MAGvK a member of the supervisory committee (DMW) and a collaborator from the University of British Columbia (HKE). Main concepts, ideas and experimental design were developed by me in discussion with MAGvK, DMW and HKE. Data were collected by HKE, 3 research assistants and me. All co-authors helped interpreting the results and edited drafts.

A version of Chapter 4 has been submitted for publication: Daros, RR; Eriksson, HK; Weary, DM and von Keyserlingk, MAG. The interplay between lameness, feeding time, and body condition changes during the dry period, and how it contributes to transition period

diseases. The paper was co-authored by my supervisor MAGvK a member of the supervisory committee (DMW) and a collaborator from the University of British Columbia (HKE). Main concepts, ideas and experimental design were developed by me in discussion with MAGvK, DMW and HKE. Data was collected by HKE, 3 research assistants and me. All co-authors helped interpreting the results and edited drafts.

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List of Abbreviations

BCS = body condition score

BHB = β-hydroxibutyrate

CI = confidence interval

DA = displaced abomasum

DIM = days in milk (or days from calving)

DMI = dry matter intake

GH = growth hormone

ICC = intra-class correlation

IGF-1 = insulin-like growth factor-1

NEB = negative energy balance

NEFA = non-esterified fatty acid

OR = odds ratio

PTH = parathyroid hormone

RP = retained placenta

SARA = sub-acute ruminal acidosis

SCK = subclinical ketosis

SD = standard deviation of the mean

TD = transition disease

TG = triglycerides

TMR = total mixed ration

VD = vaginal discharge

VLDL = very low density lipoproteins

VPC = variation partition coefficients

Acknowledgements

Thank you, Nina for the wonderful supervision and all the support through this long journey! To Dan, Maria, Stephen, José and Hanna my amazing co-authors, a huge thank you for all the discussions and contributions towards this thesis and my career, the journey would not have been as fun without you. Thank you the supervisory committee (Nina, Dan and Yvonne) for guiding me throughout my PhD and for the helpful comments and edits in the thesis.

For the participating farmers, I am honoured to have worked with you and I sincerely hope that the findings of this thesis will contribute to the welfare of your cows and the sustainability of the dairy industry in multiple countries.

To the best research assistants and interns in the world: Alexi, David, Melissa, Guilherme, Paige, Sam and Wali, you contributed immensely to this work, thank you. I hope you have enjoyed and learned as much as I did from those hard-working days.

To the research groups that I have been part of during my PhD: LETA at UFSC – Florianópolis, Brazil, Population Medicine at University Guelph – Canada and our amazing Animal Welfare Program and the Dairy Research and Education Centre at UBC – Canada, thank you a lot. To make justice to the ones I would forget to list here, I will not name all of you, but if you are reading this, be sure that you will be forever in my heart!

For all the unconditional support throughout all these years from my family, thank you so much. Bruna, thanks for being always on my side, all this would be meaningless without you.

Aos meus pais

Chapter 1: Introduction

Milk is one of the world's largest agriculture commodities; in 2016 global milk production exceeded 750 Mt, representing one of the main sources of animal protein in the world (FAOSTAT; OECD/FAO, 2017). The top 5 jurisdictions for milk production are the European Union, India, USA, China and Pakistan (OECD/FAO, 2017). Most cows are concentrated in India, China, the European Union, Pakistan, Brazil, and Ethiopia (OECD/FAO, 2017). Given dairy cattle distribution across the globe and market opportunities *e.g.*, high demand for fresh milk in India, different dairy production systems will continue to exist and will likely be adapted to the local environmental and social conditions (Thornton, 2010; OECD/FAO, 2017; Britt et al., 2018). Dairy cattle production systems are often defined as being either intensive or extensive. Intensive systems are designed to maximize milk output per cow or per area with the majority of nutrition provided as either a TMR (total mixed ration; a balanced mixture of forages, grains and minerals) or by incorporating intensive grazing as the primary forage source with some supplementation. In contrast, extensive systems are less dependent on the use of supplemental grain, relying almost entirely on grazing of natural grasslands, or kept confined where grass is manually cut and carried to the animals.

Climatic conditions between countries and regions vary, and this affects the type of production system used. For example, the long growing seasons in the tropic and sub-tropical regions favours the use of grazing, while cows in temperate regions are more likely to be housed and fed indoors (Koocheki and Gliessman, 2005; Ramankutty et al., 2008; Robinson et al., 2011; Bewley et al., 2017).

Different production systems will have unique challenges that can affect different components of animal welfare[1]. For example, cows housed indoors year-round may be exposed to uncomfortable lying surfaces, while cows kept outdoors may be exposed to rain, mud and extreme temperatures.

Longevity in dairy cattle is dependent on the cow's ability to maintain high levels of milk production, which is ultimately dependent on her ability to stay healthy and produce offspring. Calving is a traumatic event and involves a number of physiological and behavioural changes. These changes have a profound impact on energy balance and immune function (Bell, 1995; Grummer et al., 1995, 2004; Goff and Horst, 1997; Bradford et al., 2015). Unfortunately many cows are at high risk of developing diseases immediately before and during the weeks after calving (Ingvartsen et al., 2003), resulting in declines in both milk and reproductive performance and increases in culling risk – i.e. cows leaving the herd sooner than expected. This period around calving has been labelled as "the transition period" and is generally considered as the 3 weeks before to 3 weeks after calving (Grummer et al., 1995). Although the transition period has been considered important for decades (Drackley, 1999), most of the research has been done on high producing indoor housed Holstein cows, and much less research been done on grazing systems.

The introduction of this thesis will consist of four parts. Firstly, I will summarize the current understanding of the physiological and behavioural changes during the transition period, a time when cows are at most risk of succumbing to illness. Secondly, I will describe the most common transition period diseases and critically review the available literature on the main risk

[1] As defined by Fraser et al (1997), which provides a framework for animal welfare science taking into account the biological functioning (i.e. health, performance), the affective states and the animals' natural behaviour.

factors associated with these diseases. Lastly, I will compare the occurrence of transition period diseases in indoor and pasture-based systems and identify the gaps in knowledge within each of these systems.

1.1 What is transition period?

Genetics, nutrition and management of the dairy cattle are the main drivers for increased milk production in modern dairy cows (Baumgard et al., 2017). The genetic selection for milk yield has resulted in cows that have an improved ability to partition energy and protein from the diet and body reserves to support milk production instead of accumulating body mass (see review: Veerkamp, 1998). The rate of milk secretion during the first 2 – 3 mo after calving increases rapidly while the rate of dry matter intake (DMI) increases at a lower rate, reaching a maximum several weeks after peak milk production (see review: Coppock, 1985). Thus, the cow needs to be able to mobilize energy (and protein) from body reserves to compensate for the output needed to support milk production.

During the transition period cows go through major physiological and behavioural changes as they prepare to calve and begin producing milk. During pregnancy, the foetus and the placenta regulate the cow's physiology in order to maintain pregnancy, *e.g.*, suppressing the immune system, and ensuring adequate supply of nutrients to the foetus, through homeorhetic mechanisms (reviewed by Thatcher et al 1980; Eley et al., 1981). In brief, homeorhesis is a mechanism that regulates various tissues to support a physiological state, *e.g.*, the growing phase), which differs from homeostasis, a mechanism specific for maintaining physiological equilibrium (Bauman and Currie, 1980).

To prepare for parturition and the onset of lactation, a plethora of metabolic events in the days before calving trigger the onset of lactation and milk production (reviewed by Bell, 1995). These metabolic changes are concomitant with major changes in behaviour, including reduced DMI (see reviews: Grant and Albright, 1995; Sepúlveda-Varas et al., 2013); when dietary energy intake fails to supply the energy needed to support the high levels of milk production cows will experience negative energy balance (NEB; see review: Grummer, 1995). This, in conjunction with a compromised immune system (see review: Bradford et al., 2015), increases the risk of disease. Hence, the high incidence of metabolic and infectious diseases in this period (Ingvartsen et al., 2003).

1.1.1 Physiological changes during the transition period

As calving approaches increased fat mobilization and declines in DMI occur, however, NEB normally takes place after the onset of lactation (Grummer et al., 2004). Hormonal profiles are altered in the week before calving, with the majority of the acute changes taking place in the days before calving (Vazquez-Añon et al., 1994; Bell, 1995). Colostrogenesis begins 2 to 3 weeks pre-partum and is influenced by the high concentration of estrogen which facilitates the accumulation of immunoglobulins (*i.e.,* IgG-1) in the mammary gland (Brandon et al., 1971; Barrington et al., 2001). This process is suppressed by the beginning of the lactogenesis, which begins 1 to 2 days before calving, signalled by an acute drop of progesterone (P4) and exponential increase in prolactin (Gross et al., 2014; Akers, 2017). There is also a cascade of hormonal changes related to calving itself, such as an exponential increase in growth hormone (GH) and glucocorticoids (Edgerton and Hafs, 1973; Goff et al., 2002). It is not the scope of this review to describe the mechanisms of colostro- lactogenesis and calving (see Tucker, 2000 for

details), but as described below, there is a growing body of evidence linking these hormones with energy balance and immune function.

Homeorhetic mechanisms that control pregnancy and the onset of lactation increase levels of circulating GH and inhibit the production and/or tissue responsiveness to insulin and insulin-like growth factor-1 (IGF-1; De Koster and Opsomer, 2013). These hormones trigger mobilization of body reserves from adipose tissues (Tucker, 2000; Renaville et al., 2002; De Koster and Opsomer, 2013), resulting in increased circulating non-esterified fatty acids (NEFA) in blood (Adewuyi et al., 2005). NEFA can be used as an energy source in the peripheral tissues sparing glucose for milk production (Herdt, 2000). NEFA are cleared in the liver through several pathways: 1) complete oxidation; 2) partial oxidation or 3) re-esterification into triglycerides (TG; Grummer, 1993). TG are stored in the hepatocytes and exported as very-low density lipoproteins (VLDL), which can also serve as energy source in other tissues. Through partial oxidation ketones bodies are produced (acetate, BHB - β-hydroxibutyrate and acetone) and, as NEFA, can be used as energy source in other tissues. During complete oxidation NEFA enter the tricarboxylic cycle producing energy for the liver (Drackley, 1999).

The liver has limited capacity to export VLDL and to complete oxidize NEFA. Thus, NEFA are mostly metabolized through partial oxidation resulting in increased serum BHB levels (Drackley, 1999; Herdt, 2000). Cows that fail to regulate adipose tissue mobilization or fail to export VLDL from the liver are likely to develop fatty liver and/or ketosis (characterized by high levels of ketone bodies in blood - Herdt, 2000). High hepatic NEFA oxidation also decrease cows' appetite (Allen et al., 2009), contributing to NEB.

More recently the link between energy balance and immune function during the transition period has been explored. The list of hormones and metabolites and the pathways involved in

inflammation are numerous and have been extensively reviewed elsewhere (Ingvartsen and Moyes, 2013; Bradford et al., 2015; Aleri et al., 2016; Trevisi and Minuti, 2018). Increased immune response (inflammation) facilitates calving and is responsible for placental detachment (Kimura et al., 2002; Mordak and Anthony, 2015); thus, some inflammation is beneficial, but prolonged systemic inflammation is likely detrimental (Bradford et al., 2015).

During the transition period acute lipolysis stimulates a pro-inflammatory response in the adipose tissue (Contreras et al., 2015). In the liver, increased oxidation of NEFA increases the production of reactive oxygen species, which in turn stimulates the transcription of pro-inflammatory cytokines (Sordillo et al., 2009; Gessner et al., 2013; Bradford et al., 2015). After calving uterine tissues are disrupted, but the risk of pathological bacteria reaching the endometrium remains high since the cervix is still open. This is ideally counteracted by an inflammatory response that can fight infections and stimulate tissue recovery (reviewed by Chapwanya et al., 2012).

The concurrent events taking place in the adipose tissue, liver and uterus likely result in the cows experiencing systemic inflammation during the transition period (Bradford et al., 2015), which in turn require energy to mount an effective immune response (Colditz, 2002; Kvidera et al., 2017); contributing to the energy deficit. Although the link between inflammation and energy balance has not been fully elucidated, this mechanism could explain the linkage between infectious and metabolic diseases during the transition period.

1.1.2 Behavioural changes during the transition period

Several reviews have described changes in behaviour during the transition period (*e.g.* Grant and Albright, 1995; Sepúlveda-Varas et al., 2013). Feeding behaviour and DMI are the

most researched topics in this area (reviews: Grant and Albright, 2001; Hayirli and Grummer, 2004; von Keyserlingk and Weary, 2010; Beauchemin, 2018). Declines in DMI are often described to start 3 wk before calving, with the nadir taking place at calving; DMI is then thought to gradually increase post-partum until it stabilizes shortly after peak lactation (Coppock, 1985; Grummer et al., 2004). In their review, Grummer et al. (2004) looked at data from several studies to explore the pattern of DMI during the pre-partum period with the objective of describing the DMI curves for ration formulation (*e.g.*: Hayirli et al., 2003). These authors concluded that DMI starts decreasing 3 wks pre-calving with the greatest drop in DMI on the days close to calving (Hayirli et al., 2002; Grummer et al., 2004). Alternatively, in a observational retrospective study Huzzey et al. (2007) showed an association of pre-partum DMI with the development of metritis in the following lactation. Cows diagnosed with severe metritis at 6 d postpartum had lower DMI pre-partum beginning two weeks before calving while cows that remained healthy during the subsequent transition period only showed declines in DMI the day before calving. Interestingly, neither healthy or metritic cows exhibited the expected DMI decrease 3 wks before calving as proposed by Grummer et al. (2004).

Among other behaviours studied there is some evidence that restlessness, transitioning from standing and lying (i.e. standing and lying bouts), increases in the days around calving (Huzzey et al., 2005). On the day of calving cows increase total time spent standing by 2 hours (Huzzey et al., 2005). Rumination show the same pattern as DMI, dropping acutely on the day of calving and increasing gradually after calving (Kaufman et al., 2016a).

1.1.3 Management changes for transition cows

Much of the research on the transition period has focused on nutritional strategies to avoid over-conditioning (obesity) during the dry period (pre-partum) to reduce the period of NEB in the weeks following parturition, and to prevent milk fever; a prevalent acute disease caused by low levels of circulating calcium around calving. Briefly, obese cows are likely to develop "fat cow syndrome"; an affliction with a high incidence of metabolic and infectious diseases and mortality, particularly during the first 2 weeks post-partum (Morrow, 1976). To prevent obesity current recommendations suggest using low-energy diets during the dry period (NRC, 2001). However, when low-energy diets are fed pre-partum cows have lower DMI (Hayirli et al., 2002), thus some have argued that higher energy diets should be used pre-partum to compensate and prevent the detrimental effects of a long period of NEB. Today, feeding high energy diets (as a means to increase DMI) during the pre-partum phase are known to be associated with a greater depression in DMI before calving and lower DMI after calving compared to cows fed diets to meet their nutrient requirements (Janovick and Drackley, 2010).

Increased understanding of the role of parathyroid hormone (PTH), vitamin D and blood pH on calcium metabolism has led to the development of advanced diets fed to pregnant dairy cattle, high in anions, so called "DCAD diets", and also diets low in calcium (reviewed by Goff, 2006). These diets decrease blood pH, causing metabolic acidification, this in turn increases tissues responsiveness to PTH, which stimulates calcium mobilization from bones and increases intestinal calcium absorption (Horst et al., 1997; Goff, 2008). The use of these diets has dramatically reduced the incidence of milk fever; incidence of milk fever across USA is lower than 3% (USDA, 2016). The effects of using DCAD diets for the whole dry period remains controversial (DeGaris et al., 2010; Weich et al., 2013; Wu et al., 2014; Lopera et al., 2018).

To facilitate feeding different diets during the dry period in indoor housing systems cows are often subjected to regrouping according to their calving date, with the first regrouping taking place 3 to 4 weeks pre-partum. In the days leading up to parturition cows are moved to the maternity pen where she will calve and then moved to the hospital or "fresh" pen for several days until she is regrouped with the main lactating herd. In contrast, most pasture systems keep dry cows as a single group that may or may not receive dietary supplements as calving approaches.

The impacts of regrouping, stocking density at the feed bunk and stall in relation to changes in behaviour, immunity and transition period diseases in indoor systems have all received considerable attention (see reviews by Sepúlveda-Varas et al., 2013; Proudfoot and Habing, 2015; Chebel et al., 2016). However, little research has focused on the management of cows during the transition in grazing systems (see review: Kay et al., 2015). I further discuss this literature on a section below.

1.2 The transition period diseases

As discussed previously, the early post-partum period is when cows are at greatest risk for metabolic and infectious diseases. Below I describe the most common diseases that are highly prevalent in dairy herds.

1.2.1 Metritis

The infection of uterine epithelia is considered metritis and is associated with reduced reproductive performance and milk production (see review: LeBlanc, 2008). Metritis usually occurs in the first 2 weeks after calving; clinical signs including watery foul vaginal discharge

(VD), fever (>39.5C), dullness or other signs of systemic illness (Sheldon et al., 2006). When all signs of metritis are present it is normally referred to as acute puerperal metritis; clinical metritis is usually used when only foul VD and no signs of systemic illness are present (Sheldon et al., 2006). For the purposes of this review both categories are regarded as metritis.

Metritis is a painful disease (Stojkov et al., 2015) caused mainly by bacteria present in the environment, such as, *Arcanobacterium pyogenes, Escherichia coli and Fusobacterium necrophorum* (Sheldon et al., 2006). The relaxation of the physical barriers (i.e. cervix and vagina) that normally prevent bacterial contamination to the uterus cause most cows to test positive for bacterial culture in the first weeks post-partum (Chapwanya et al., 2012), despite not all cows exhibiting clinical signs. Metritis occurrence is dependent on the cow's ability to mount an effective immune response to fight the infection (LeBlanc, 2014).

1.2.2 Retained placenta

Failure to detach foetal membranes within 24h of calving is considered RP and is usually diagnosed by visual inspection of the vulva (Kelton et al., 1998). RP was thought to be a result of failure to expel the foetal membranes due to lack of uterine motility (see review: Laven and Peters, 1996). More recent evidence suggests that RP is caused by a failure to mount an effective immune response needed for the breakdown of the cotyledon–caruncle attachment (reviewed by LeBlanc, 2008). The function of some cells of the immune system has also been shown to be impaired when there are low levels of intracellular calcium (Kimura et al., 2006), hence the association between milk fever and RP. Metritis and RP share common pathophysiology through reduced immune function, so there is a high likelihood that cows with RP will subsequently develop metritis (*e.g.*, Dubuc et al., 2010).

1.2.3 Subclinical ketosis

Rapid lipid mobilization around calving overwhelms the liver capacity to completely oxidize, re-esterify and export triglycerides (TG) from the liver, resulting in partial oxidation of NEFA, generating ketone molecules (acetoacetate, BHB and acetone). Ketone molecules can be used as an energy source in the peripheral tissues and are part of the glucose-sparing mechanism present in early lactation – glucose in early lactation is usually taken up by the mammary gland to produce milk (Goff and Horst, 1997). Consequently, ketosis (or hyperketonaemia) results from a maladaptive mechanism of the adipose tissue and liver metabolism.

Ketosis has been categorized in either Type I or II (Holtenius and Holtenius, 1996). Type I ketosis refers to low glucose and low insulin levels caused primarily by severe negative energy balance (NEB). In contrast, Type II ketosis is a consequence of an impairment in insulin signalling and responsiveness; insulin resistance resulting in continuous fat mobilization (i.e. continues to export NEFA) (reviewed by Herdt, 2000). The liver limited capacity to completely oxidize NEFA increases secretion of ketone molecules in the hepatic cell cytosol which are then converted to BHB and released into the bloodstream (Drackley, 1999; Herdt, 2000). Type II ketosis also increases the chances of fatty liver development, as normal levels of glycogen in the blood allow the activation of the re-esterification pathway, where NEFA are reverted to TG and stored in the hepatocytes (Drackley, 1999; Herdt, 2000). Type II ketosis occurs in the first weeks after calving as the homeorhetic mechanism to support the start of lactogenesis induces insulin resistance (Herdt, 2000). In indoor dairy production systems, Type II ketosis predominates (Holtenius and Holtenius, 1996); as post-partum diets are formulated to provide for the increased energy demand thereby decreasing the chances of Type I ketosis.

Diagnosis of clinical ketosis is often difficult as it involves subjective measurements, including depressed appetite and the absence of other clinical disease (Duffield et al., 1999). Validated cow-side tests for levels of circulating ketone molecules (reviewed by Tatone et al., 2016) have improved the ability to detect subclinical ketosis (SCK). The common threshold used to define SCK is 1.2mmol/L of BHB (LeBlanc, 2010). Another authors suggested that different threshold varying from 1.0 to 1.4mmol/L depending on the type of disease evaluated to be associated with BHB (Oetzel, 2004; Duffield et al., 2009; Ospina et al., 2010). Furthermore, almost without exception, these studies have been done on high producing Holsteins cows housed under zero grazing systems, thus the application of same thresholds for outdoor systems and breeds must be used with caution.

1.2.4 Other common diseases

Mastitis is the result of intra-mammary infection, usually caused by bacteria (reviewed by Ruegg, 2017). Mastitis, unlike metritis and RP, can occur any time during lactation and the dry period (Barkema et al., 1998). However, the incidence of mastitis is greatest during the transition period, irrespective of whether cows are housed on pasture (Petrovski et al., 2009) or indoors (Olde Riekerink et al., 2008).

Hypocalcaemia, also known as milk fever or parturient paresis, usually happens within days of calving due to failure of the homeostasis mechanisms to support high calcium demand for milk production (reviewed by Goff, 2008). Cows with milk fever become recumbent and if not treated promptly the chances of recovery decreases dramatically (Green et al., 2008; Stojkov et al., 2016) The prevention of clinical hypocalcaemia is now argued to be highly dependent on nutrition management (see review: Murray et al., 2008). Despite advances in nutrition,

subclinical hypocalcaemia is still prevalent (Reinhardt et al., 2011; Venjakob et al., 2017). Recent epidemiological studies have found associations of sub-clinical hypocalcaemia with immune function, health and reproductive parameters (*e.g.*, Martinez et al., 2012; Ribeiro et al., 2013) and this has been suggested as one of the possible mechanism of which other transition period diseases may arise.

1.3 Risk factors for transition period diseases

Risk factor is a general term used to describe any factor that may be associated (positively or negatively) to disease occurrence. Below I review the most important risk factors for the main transition period diseases; RP, metritis and SCK. The risk factors will be separated into cow- and herd-level factors. Where available, controlled studies that have focused on the causal relationship between risk factor and disease will be highlighted.

1.3.1 Cow-level factors

1.3.1.1 Body condition score

BCS is correlated with the amount of adipose tissue (Gregory et al., 1998), making it useful for assessing cow's condition regarding its long term nutritional status. Different BCS scoring systems are available, but all broadly differentiate between cows that are thin, moderate or obese (Roche et al., 2004). From calving to peak lactation (usually from 50 to 100 days in milk - DIM) BCS changes dramatically, with cows losing condition due to the NEB experienced after parturition. The controlling mechanisms of body fat mobilization include both homeorhetic mechanisms (described above) which are mainly dependent on genetic traits (*e.g.*, Zachut and

Moallem, 2017) and homeostatic mechanisms, that are highly influenced by environmental factors, such as type of diet (see review: Roche et al., 2009). The BCS at calving, nadir BCS in early lactation, time from calving to nadir BCS and, amount of BCS lost from calving to nadir have all been associated with lower productive and reproductive performance, and disease occurrence (reviwed by Roche et al., 2009). BCS at calving is associated with nadir BCS and amount of BCS loss in early lactation (Chebel et al., 2018) making this parameter useful to predict cows at risk of developing transition period disease (Roche et al., 2009).

Regardless of type of housing, high BCS at calving is associated with metabolic diseases, especially ketosis (*e.g.*, Vanholder et al., 2015), while low BCS at calving is associated with uterine diseases, mainly RP and metritis (*e.g.*, Duffield et al., 2009). In controlled studies, high BCS cows reduce DMI after calving (Hayirli et al., 2002) and lose more weight and body condition in the weeks after calving (Roche et al., 2013b). Under experimental conditions, fatter cows had lower activation of immune function related genes (Crookenden et al., 2017), possibly providing some explanation as to the mechanism between high fat mobilization and decreased immune function. Conversely, the causal link between low BCS at calving and uterine diseases remains unknown. It could be speculated that low BCS reflects a current subclinical disease, but more work is needed to address this hypothesis. Alternatively, some work has shown that the amount of BCS lost from dry-off to calving was associated with uterine health (RP and metritis) compared to cows that did not lose BCS in the same period (Markusfeld et al., 1997; Chebel et al., 2018). During the dry period fatter cows lose more body condition (Roche et al., 2009; Chebel et al., 2018). In summary, managing BCS during lactation to enter the dry period to avoid obesity should decrease the incidence of both metabolic and infectious diseases. This idea is supported by some work on grazing cows in which researchers assessed the effect of dietary

management to achieve targeted BCS during the dry period, and found that treatments where cows had medium BCS during the dry period had lower NEFA and BHB levels compared to cows managed to have higher BCS (Roche et al., 2013b, 2015).

1.3.1.2 Breed

Few studies have found breed to be a risk factor for transition period diseases. In Canada, in free-stall and tie-stall systems, Jersey cows were found to have a higher prevalence of SCK compared to Holsteins cows (Tatone et al., 2017). Conversely, in two seasonal calving pasture based dairies in Florida, Jersey cows had a lower prevalence of SCK but a higher prevalence of subclinical hypocalcaemia compared to Holstein cows (Ribeiro et al., 2013). However, not all studies report breed differences. A large multi-country study from Europe found no evidence of differences in ketosis incidence across breeds (Berge and Vertenten, 2014). However, it should be noted that indoor housing systems tend to have only a single breed, thus farm becomes a confounding factor making it difficult to draw conclusions about breed difference from such studies. Ideally, differences across breeds should be studied in herds that use multiple breeds (*e.g.*, Ribeiro et al., 2013).

Why disease incidence should vary across breeds is not clear. It has been suggested that Jersey cows have lower levels of $1,25(OH)_2D$ receptors in the intestines compared to Holsteins cows, making Jerseys more susceptible to hypocalcaemia (Goff, 2014). A series of studies in New Zealand and Ireland used different strains of Holstein-Friesian cows (North American Holstein *vs* New Zealand Holsteins); not surprisingly, different strains responded differently in different production systems, however, in these studies researches only compared production and reproductive variables (see reviews: Baudracco et al., 2010; Abdelsayed et al., 2015).

1.3.1.3 Parity

In general primiparous cows have higher incidence of uterine diseases and lower metabolic diseases during the transition period when compared to multiparous cows (*e.g.*, Giuliodori et al., 2013; Tatone et al., 2017). In most dairy production systems heifers usually calve around 24 months of age, increasing the chances of calving difficulty (i.e. dystocia), due to a narrower birth canal compared to fully grown cows (reviewed by Mee, 2008). Dystocia has been reported across different production systems and breeds and results in increases in RP and metritis occurrence. Interestingly, studies were able to identify a quadratic relationship between parity and metritis, i.e. primiparous and older cows (>3 lactations) were at more risk of developing metritis (Bruun et al., 2002). Neutrophil function of older cows is impaired compared to younger cows (Gilbert et al., 1993) which may explain their higher susceptibility to infections. Multiparous cows have reduced numbers of PTH and $1,25(OH)_2D$ receptors in the kidney and intestines, respectively, hence the higher incidence of milk fever in older cows (Goff, 2014). To my knowledge no study has assessed why multiparous cows have a higher prevalence of SCK compared to primiparous cows. However, in indoor housed dairy cows the dynamics of BHB are different between parity groups, with primiparous cows having higher BHB at the beginning of the lactation with a gradual decline while multiparous cows show an continuous increase from the day of calving until it peaks at 9 -11 DIM and then follow a gradual decrease in BHB (Santschi et al., 2016; Tatone et al., 2017). This may be explained in part by primiparous cows producing less milk, but it cannot explain this phenomenon entirely given that these cows also eat less and require additional energy for growth compared to multiparous cows. Further research is warranted to understand the reasons for of the difference in SCK in primiparous compared to multiparous cows.

1.3.1.4 Age at first calving, gestation and dry period length

Heifers that calve at 25 months of age or older, and cows having longer dry periods (>70 days), both have higher levels of SCK (Tatone et al., 2017). These factors have also been associated with higher BCS at calving (reviewed by Roche et al., 2009). Some experimental research has manipulated dry period length from 0 to 60 days dry. Unfortunately the findings to date are inconclusive with the exception that prolonging lactation until calving (i.e. no dry period) seems to improve metabolic health (van Knegsel et al., 2014; van Hoeij et al., 2017) without detrimental effects on uterine health post-partum (Chen et al., 2017). These studies have been performed in indoor systems using high producing Holsteins and thus may not extend to lower producing cows in pasture systems. There have been no reports investigating dry period length on metabolic diseases and metritis in pasture based systems, however a longer dry period is associated with higher incidence of clinical mastitis in the first few weeks after calving in grazing herds in New Zealand (Bates and Dohoo, 2016).

Shorter gestation length has been associated with higher incidences of RP (Muller and Owens, 1974); it is likely that in shortened gestations the placentomes are not fully matured (Laven and Peters, 1996) and the hormonal balance required for placental detachment is not fully in place (Beagley et al., 2010). Heat stress is associated with short gestations (Tao and Dahl, 2013); however, RP incidences are higher during cooler months across different production systems (*e.g.*, Quiroz-Rocha et al., 2009). More details on the seasonal effect on diseases incidence are provided below.

1.3.1.5 Milk yield and milk components

There is no evidence of an association of milk production or components on the likelihood of cows developing metritis or RP (Ingvartsen et al., 2003). Tatone et al., (2017)

found that herd level milk production and milk fat percentage before dry-off were both negatively associated with SCK. It is likely that herds that produce more milk also have better general management and thus may have implemented ketosis prevention protocols.

1.3.1.6 Physiological parameters

The search for a simple, cheap and easy way to measure physiological parameters with good predictive value for transition period diseases continues (see review: Overton et al., 2017). As discussed previously, circulating NEFA levels can serve as a proxy for changes in energy balance during the transition period (*e.g.*, Dubuc et al., 2010; Ospina et al., 2010), with SCK showing the strongest correlation with NEFA (review by Ospina et al., 2013). Unfortunately, to date there is no cow-side test for NEFA assessment (Overton et al., 2017). Serum or milk BHB have both been successfully used to identify cows at risk for clinical ketosis, displacement abomasum and uterine disease, however the predictive value of BHB for transition period diseases is higher during the post-partum period (Overton et al., 2017), when it is often too late to implement prevention strategies. However, the availability and reliability of cow-side tests for BHB make this a powerful tool for herd diagnostics that can improve transition period management (Ospina et al., 2013).

There are a few other markers that have shown promise as predictors of transition period disease. General inflammation markers, such as haptoglobin have been associated with transition period diseases. However, the predictive value of these markers are greater during the post-partum period (Dubuc et al., 2010; Huzzey et al., 2015). Studies investigating metabolomics and proteomics have identified a range of parameters associated with metabolic and infectious diseases (*e.g.*, Dervishi et al., 2016b; Zhang et al., 2017). Interestingly, parameters associated with immune function and inflammation were different between healthy and affected cows in the

post-partum period as much as 8 weeks before calving (Dervishi et al., 2016a; Trevisi and Minuti, 2018). Although metabolomic studies are promising, the work to date suffers from poor replication with the majority of studies making use of very few animals (Dervishi et al., 2017; Zhang et al., 2017).

Italian researchers have proposed the use of indexes, such as the liver activity index, liver functionality index and post-partum inflammatory response index, to categorize cows at risk for transition period disease (reviwed by Trevisi and Minuti, 2018). These indexes combine several blood inflammation markers and have been associated with lower reproduction performance. Although these indexes were designed for post-partum measurements, recent findings of a variety of markers during the dry period associated with transition period health indicate the possibility for the development of new indexes (Trevisi and Minuti, 2018). Most of this research is limited to a few herds in Italy, so the use of these indexes across herds should be viewed with caution, especially because immune function markers may be herd dependent (Zecconi et al., 2018).

1.3.1.7 Behaviours and DMI

Reductions in DMI pre-partum have been reported for many years (*e.g.*: Coppock et al., 1972; Johnson and Otterby, 1981). DMI is a function of feeding behaviour (see Nielsen, 1999); in brief, DMI is a function of feeding rate and total time spent feeding. Although the relationship between feeding time and DMI is not perfect, and impacted by illness (Huzzey et al., 2007), measures of feeding behaviour have been used to detect and predict disease risk in beef cattle (Sowell et al., 1998; Quimby et al., 2001). In dairy cows, feeding behaviour has been used to identify cows at risk for metritis (Urton et al., 2005; Huzzey et al., 2007) and at risk for clinical and SCK (González et al., 2008; Goldhawk et al., 2009). In these studies cows that were disease

positive had lower daily feeding times during the pre-partum period. Conversely, DMI was higher in the weeks pre-partum for cows that developed subclinical hypocalcaemia compared to cows that had normal levels of calcium on the day of calving; daily feeding time was not reported (Jawor et al., 2012).

Schirmann et al. (2016) also showed decreased DMI and total feeding time per day during the pre-partum period but only for cows that were diagnosed with metritis and SCK at the same time (defined as serum BHB ≥ 1.2mmol/L) and not for cows diagnosed with metritis only. More recent work has shown that the relationship between feeding behaviour and illness in dairy cows is not straightforward. Recent work by Neave et al. (2018), did not find any association between feeding behaviour before calving for cows diagnosed with metritis compared to cows without metritis. However, cows did show decreased feeding behaviour (*i.e.*: DMI and number of meals per day) in the days before diagnosis. Furthermore, these differences were exacerbated when cows had metritis and SCK at the same time (Neave et al., 2018). The latter two studies contrast with the findings of Huzzey et al. (2007), which did not assess BHB level. Furthermore, DMI may be intrinsically correlated with levels of circulating NEFA (Allen et al., 2009), which has been described as a major risk factor for ketosis and metritis (Ospina et al., 2010). In fact, this relation between NEFA, DMI, BHB and metritis has been previously described by Hammon et al. (2006) who stated that high NEFA levels in the week pre-partum were associated with decreased DMI, which in turn was associated with lower immune function and the development of metritis and sub-clinical ketosis (Hammon et al., 2006).

Standing and lying behaviour during the pre-partum period have been associated with dystocia (Proudfoot et al., 2009a), subclinical hypocalcaemia (Jawor et al., 2012), metritis (Neave et al., 2018) and ketosis (Itle et al., 2015) but not SCK (Kaufman et al., 2016b). The

direction in which standing and lying behaviour change before calving may be disease specific. For example, cows diagnosed with dystocia exhibit more standing/lying bouts during the day of calving. While other diseases, such as ketosis and metritis, changes in standing/lying behaviour before calving may not be a cause of the post-partum condition but instead reflect an indirect association. It has been speculated that cows that develop ketosis may have been of low social rank (i.e. subordinate animals) thus avoiding agonistic interactions at the feed bunk and waiting for longer to feed (Itle et al., 2015).

Several types of commercial activity monitors are now available that are designed to assist farmers with oestrus and disease detection (reviwed by Rutten et al., 2013). These technologies have been proven useful to detect transition period diseases (*e.g.* Stangaferro et al., 2016) and are often based on a combination of changes in activity and rumination. However, the algorithms used to generate health-monitoring alerts on these systems are often not publicly available impairing reproducibility. Also, different activity monitoring systems can show poor agreement in some of the parameters analysed (Nielsen et al., 2018). Nonetheless, I see much promise in these systems to help farmers make evidence based decisions (*e.g.* Stangaferro et al., 2016).

1.3.2 Herd-level factors

1.3.2.1 Management factors

Cows are a social species, show synchronized behaviours and have a complex social hierarchy (DeVries et al., 2003; Val-Laillet et al., 2008). Regrouping and overcrowding have similar effect on behaviour of dairy cows, increasing agonistic interactions and disrupting

feeding and lying behaviour (Schirmann et al., 2011). Regrouping is an acute stressor, *i.e.* there is a sharp increase in agonistic interactions in the first day following regrouping that quickly resume to baseline levels after few days (von Keyserlingk et al., 2008). Conversely, overcrowding is a more chronic stressor as cows take longer to adapt (Proudfoot et al., 2009b). Overcrowding has also been associated with increased levels of glucocorticoids (Huzzey et al., 2012; Fustini et al., 2017) suggesting that overcrowding during the pre-partum period may be particularly detrimental to cow health. The impact of overcrowding cows has been shown to be much larger in subordinate than in more dominant cows (Huzzey et al., 2012). The impact of regrouping and stocking density in physiological parameters, disease incidence, reproductive and milk performance was recently reviewed by Chebel et al. (2016). Weekly regroupings during the dry period did not affect energy and immune status, reproductive and production performance or disease incidence compared to cows that stayed on a stable group throughout the dry period (Silva et al., 2013b; a). The effect of stocking density (80 *vs* 100%) during the dry period was investigated using cows housed at each stocking rate and reported differences in lying and feeding behaviour on the week before calving (Lobeck-Luchterhand et al., 2015); however, these changes did not translate into higher incidence of post-partum diseases (Luchterhand et al., 2016). However, these studies should be viewed with caution since treatment was applied at a single group thus suffered from pseudo-replication.

Few other management practices have been associated with transition period disease. Early mastitis incidence (clinical cases within 30 of calving) seem to be highly affected by different management strategies during the dry period, such as dry cow therapy strategies and cleaning routine (Green et al., 2007). Cows housed on pasture during the dry period benefit from rotational grazing schemes, possibly by having cleaner lying surfaces while cows kept indoors

benefit from routine stall and calving pen cleaning (Green et al., 2007). These results highlight the importance of clean lying surfaces to control the incidence of mastitis during the dry and post-partum periods.

A single study has reported that cows in herds with no access to pasture have higher odds of developing metritis (Bruun et al., 2002). Conversely, another study reported that cows allowed to graze in the summer have a higher prevalence of SCK (Berge and Vertenten, 2014). An experimental study by Olmos et al. (2009), comparing health parameters between housed and grazing cows, showed that grazing cows had higher BHB and lower rumen fill compared to housed cows. Epidemiological studies on differences in disease incidence between production systems are likely confounded by other unmeasured management practices, such as diet type, or cow-level factors such as parity or BCS therefore, generalizations from these findings are difficult to be made.

Experimental studies focusing on the effects of management practices on disease incidence are difficult to perform, it requires interventions that last for long periods, the number of cows required is high, and involves an intensive cooperation with farmers. Alternatively, epidemiological studies require a high number of farms to capture the variability of management practices with enough replication (i.e. enough farms using the same management practice) to allow for testing. Large epidemiological research has been carried in Europe and North America (*e.g.*, Bruun et al., 2002; Chapinal et al., 2011; Suthar et al., 2013; Berge and Vertenten, 2014; Tatone et al., 2017), where most of cows are kept indoors. To my knowledge, large studies have not yet been performed on pasture-based systems.

1.3.2.2 Ambient factors

There are seasonal effects on transition period disease incidence. In Europe, during the spring months SCK incidence tends to be higher than in winter months (Suthar et al., 2013; Berge and Vertenten, 2014; Vanholder et al., 2015). In Canada there is a high incidence of SCK during May but not, during the other spring months (Tatone et al., 2017). Season is also a risk factor for metritis and RP; with higher incidences during the winter months (Muller and Owens, 1974; Bruun et al., 2002; Quiroz-Rocha et al., 2009; Chapinal et al., 2011). In year-round calving pasture systems that are no reports of an effect of season on transition period diseases incidence. However, rainfall at calving is associated with higher odds of clinical mastitis in the first 90 days post-partum in grazing systems in New Zealand (Bates and Dohoo, 2016).

1.3.3 Associations between different diseases during the transition period

As discussed previously, early fat mobilization and inflammation can lead to a myriad of diseases. The increased levels of NEFA overwhelms the liver capacity to metabolize fat which further impairs liver function causing fatty liver, SCK, and may lead to the development of clinical ketosis and displaced abomasum (DA; reviewed by LeBlanc, 2010). Moreover, high levels of circulating BHB and liver inflammation negatively affects the immune system increasing susceptibility to infectious diseases such as metritis and mastitis. At the same time, the lack of calcium impairs immune system functionality leading to RP (Kimura et al., 2002, 2006). Recent research has shown that there is a high energetic cost to mount an inflammatory response (Kvidera et al., 2017), further exacerbating NEB. Moreover, the sudden change from high-fibre/low-energy to low-fibre/high-energy diets that is common around calving is thought to contribute to sub-acute ruminal acidosis (SARA), which in turn increase rumen wall

permeability ("leaky gut"), allowing endotoxins to enter the bloodstream unchaining an inflammatory response (reviewed by Zebeli et al., 2015). Together this highlights the complexity of events and how one disease may contribute to another during the transition period.

Lameness has been considered one of the main health problems affecting indoor housed cows (von Keyserlingk et al., 2012; Solano et al., 2015; Randall et al., 2019) but recently some studies have also shown lameness to be prevalent in some grazing dairy herds (Ranjbar et al., 2016; Bran et al., 2018) but not in others (Fabian et al., 2014). Lameness can be caused by infectious or non-infectious lesion (Tadich et al., 2010). In brief, infectious lesions usually appear as digital dermatitis, which are associated with increased levels of contact to wet contaminated sources of the pathogens (Palmer and O'Connell, 2015). Non-infectious lesions are usually caused by trauma, arising from punctures or by chronic exposure to hard surfaces (Bicalho and Oikonomou, 2013). The most common non-infectious claw lesions are sole ulcers and white line disease (Murray et al., 1996). Recently, the thickness of the digital cushion has been associated with the development of non-infectious claw lesion which, in turn, is highly dependent on BCS (Machado et al., 2011). This finding highlights the importance of BCS management, not only for metabolic and infectious disease, but also for claw lesions.

Despite the amount of work done on lameness of lactating cows, only a few studies have addressed the link between lameness and the transition period diseases, or even the cure and incidence rates of lameness during the dry period. One study on free-stall housed cows in New York State described the prevalence of cows diagnosed with claw lesions (sole ulcers and white line disease) at dry off – 26% (Machado et al., 2011). Unfortunately, lameness prevalence was not reported in this study.

To my knowledge only 2 studies assessed the effect of lameness during the dry period on the occurrence of transition period diseases in indoor housed cows. Calderon and Cook (2011) followed lame cows between 1 to 3 weeks before calving and found that they had higher blood levels of BHB compared to non-lame cows. These authors also reported that lame multiparous cows in the close-up period lay down for longer and speculated that these cows might have traded feeding time for lying time. Indeed, changes in feeding and lying behaviour may be the obvious link between lameness and transition period disease. For instance, multiple studies on indoor-housed cows report that lame cows lie down for longer, spend less time feeding and have lower DMI when compared to non-lame counterparts (Bach et al., 2007; Ito et al., 2010; Miguel-Pacheco et al., 2014). Lameness prevalence in the 2 weeks before calving was reported to be 11.2% and was associated with increased risk of cows being treated for transition period diseases within 30 after calving (Vergara et al., 2014). However, this result should be interpreted with caution as there were interactions between lameness and parity and calving abnormalities.

Lameness is also associated with inflammation markers. Lame cows have higher haptoglobin (Smith et al., 2010; O'Driscoll et al., 2015). Tadich et al., (2013), showed that haptoglobin levels increased as gait score (scale 1 to 5) increased. No studies to date have explored the association between lameness and inflammation as a causal pathway for transition period disease.

1.4 Production systems and transition period diseases

Cows can adjust to different housing systems. The main dairy cattle housing systems can be divided as indoor and outdoor housing. The former includes tie-stalls, free-stalls and loose housing systems while the latter includes pasture-based systems. A brief description of such

systems follows. A summary of the occurrence[2] of the most common transition period diseases between indoor and pasture-based production systems is provided on Table 1.1.

Table 1.1. Occurrence of common transition period diseases by housing systems.

Disease[1]	Production system[2]	
	Indoor	Pasture
Metritis[3]		
n of studies[4]	5	1
Mean incidence	20.9	17.3
Range	16.7 – 29.7	-
n of studies[4]	1	2
Mean prevalence	18.7	7.7
Range	-	5.3 – 11.2
RP[5]		
n of studies[4]	3	4
Mean incidence	13.6	4.1
Range	8.9 – 18.7	1.7 – 13.9
SCK[6]		
n of studies[4]	4	2
Mean incidence	37.5	49
Range	19.7 – 43.2	16.6 – 66.5
n of studies[4]	12	5
Mean prevalence	24	24.8
Range	10 – 58.8	10.8 – 35.4

[1] The mean of disease incidence and prevalence was weighted by the number of cows assessed in each study.
[2] Indoor: Include studies on tie-stall, free-stall and other loose housing systems. Pasture: include seasonal and year-round rotational grazing systems.
[3] A range of metritis definition was found on the assessed studies and included: foul watery VD with or without fever and with or without signs of systemic illness.
[4] A detailed list of each study is provided on Appendix A.
[5] RP defined as failure to pass foetal membranes within 12 or 24 h after calving.
[6] SCK defined as blood BHB > 0.96mmol/L or milk BHB > 0.15 mmol/L from 0 to 21 DIM.

1.4.1 Production systems

1.4.1.1 Indoor housing systems

These systems encompass the tie-stalls, free-stalls and other loose housing systems. Tie-stalls systems were primarily developed to house cows for some period of the year (i.e. winter) in small-scale farms (less than 100 cows) located in temperate countries (reviewed by Bewley et al.,

[2] The term occurrence represents the prevalence and incidence of diseases. Incidence measures require longitudinal studies while prevalence can be obtained from cross sectional studies.

2017; see also: Bickert and Light, 1982). Cows housed in tie stalls are tied using rope or chain by their neck to individual stalls where that are provided feed and water. Some tie-stall barns have "exercise pens" (i.e. a dirt lot or a pen with concrete flooring), where cows can spend parts of the day free to explore and interact (Keil et al., 2006; Popescu et al., 2013).

The use of tie-stall has declined in many countries (Barkema et al., 2015), partially driven by increases in herd size but, more recently, due to public pressure. In Norway and Sweden the construction of tie-stall barns was outlawed since 2004 and 2007, respectively, and the European Union has recognized that tie-stalls impose hazards to the welfare of cows due restriction of movement (Algers et al., 2009). This seems to be in agreement with the public, which in general disavow production of animals in confinement (Schuppli et al., 2014; Spooner et al., 2014; see also: Weary et al., 2015). Therefore tie-stall use will likely decrease in the coming years.

Free-stalls allow cows to roam but delineates the lying space for each cow. They were introduced to accommodate the increasing herd size in more developed countries (Bickert and Light, 1982). Free-stalls are the most common housing system for herds of more than 500 cows in USA (USDA, 2010) and has been increasingly used in some European countries. The surface where cows lay down may vary from plain rubber mats to deep-bedded stalls with sand, wood shavings or dried manure (USDA, 2016). Alleyways are usually cemented, although some farms use rubber flooring to improve cow comfort (USDA, 2016). Stalls dimensions and hardware regarding cow comfort and stall cleanliness have been researched extensively (in free- and tie-stall barns: e.g., Tucker et al., 2004, 2005; Zurbrigg et al., 2005; Bouffard et al., 2017). In brief, the stall needs to provide enough space for the cow to stand up and get up freely. The neck rail and brisket board are designed to prevent the cow from standing too far forward to avoid manure deposit on the stall. In free-stall systems cows are milked at least twice a day. Barns are usually

equipped with curtains to prevent air drifts during the winter and allow airflow during the summer, and fans and sprinklers are often used to reduce heat stress (reviewed by Collier et al., 2006). Manure is handled mechanically through the use of automatic scrappers, robotic cleaners, skid tractors or by water (i.e. flush barns – alleys are inclined to facilitate flow as water is flushed down routinely). Cows are grouped in pens to facilitate the distribution of balanced diets according to the group's need (Grant and Albright, 2001). Feed is offered *ad-libitum* at the feed-bunk. Considerable research has been done on the effects of feed barrier design on cow's behaviour (*e.g.*, Endres et al., 2005; DeVries and von Keyserlingk, 2006).

Open pack systems (i.e. pens with soft bedding without stalls) are an alternative to free and tie-stall systems. Open packs are usually used in small herds or as part of large free-stalls farms to house specific animals (*e.g.*, heifers, dry cow, fresh or sick cows - Espadamala et al., 2016; Fogsgaard et al., 2016; Costa et al., 2018). The benefit for the cows in these systems is the soft lying surfaces. However, softness and cleanliness are dependent on bedding material and the management. Some argue that the cost to maintain clean bedding are prohibitive, hence the increased popularity of compost bedding systems, so called "compost barns" (see review: Bewley et al., 2017). In compost barns, the bedding is aerated multiple times per day to allow aerobic fermentation (i.e. composting), controlling bacterial growth without the need of adding/removing bedding material. The efficiency of the composting process in reducing pathological organisms is influenced by the humidity of the bedding, which is a function of area per cow and air humidity (Black et al., 2014), so managing compost barns can be a challenge in very humid climates. In open pack systems the feeding management is similar to that applied on free-stalls.

1.4.1.2 Intensive pasture-based systems

These systems are common in traditional dairy regions where the climate allows for long or year-round grass growing seasons. New Zealand, the biggest milk exporter in the world, produces most of its milk from intensively managed grazing farms (Roche et al., 2017). Ireland (Läpple et al., 2012) and northeast US also use intensive grazing systems for parts of the year – in the US and Europe this system is very popular among organic producers. Year-round intensive pasture systems are common in subtropical areas of South America (Cappellini, 2011; Balcão et al., 2017).

Two types of intensive pasture system can be differentiated: management intensive grazing and mixed feeding grazing. The first one relies on the majority of the feed from direct grazing, thus the pastures areas need to be managed intensively to allow high production of forage. This is usually achieved by dividing the pasture areas in multiple paddocks rotating the grazing herds from one paddock to next often enough so the cows are not allowed to graze the re-growth of that grass until it reaches optimal growth (Voisin, 1959; Roche et al., 2017). Some intensive dairies rotate the grazing herds every milking to ensure cows are eating the highest grass quality; however, some supplementation is used to achieve high milk yields (see review: Knaus, 2016). On mixed feeding cows are supplemented with silage and grain in the feeding barn and are allowed to graze after being fed.

1.4.2 General health in different production systems

As reviewed above (see also Table 1.1), there is little evidence that disease occurrence is different between production systems. A recent review (Arnott et al. 2017) concluded that the occurrence of production diseases was lower when cows had access to pasture, but it is worth

noting that: 1) this was not a systematic review and, 2) most of the studies cited were from countries where year-round pasture housing is not possible. A recent study in 53 dairies using year-round intensive pasture-based systems reported that 30% of the cows were diagnosed as lame (Bran et al., 2018), while a study in year-round grazing cows reported that 26% of cows developed ketosis in the first 6 weeks after calving (Garzón-Audor and Oliver-Espinosa, 2019). Together these results indicate that in some herds the occurrence of diseases in grazing cattle is high.

Lameness is considered to be one of the greatest welfare challenges facing indoor housed cow (Huxley, 2013). This malady affects over a quarter of cows housed indoors (*e.g.*, von Keyserlingk et al., 2012; Solano et al., 2015; Randall et al., 2019) and has being associated with poor lying surfaces and long standing times on hard surfaces (Ito et al., 2010). Although traditionally viewed as a problem for indoor housed cows, recent work has shown that lameness prevalence's is also high in pasture based systems (e.g., Ranjbar et al., 2016; Bran et al., 2018). In free-stall systems, facility design features such as the use of mattresses and lack of bedding have been identified as risk factors for lameness (Chapinal et al., 2014; Solano et al., 2015). In outdoor systems, lameness seems to be related to routine herd management such as, how the cows are moved and whether cows receive preventive hoof trimming or not (Ranjbar et al., 2016; Bran et al., 2018). Therefore, different strategies may be required to reduce lameness prevalence in indoor and outdoor systems.

1.5 Conclusions and thesis aims

The literature reviewed here shows major physiological and behavioural changes in relation to calving and the association between these changes and disease. For example, early

reductions in DMI (Huzzey et al., 2007), loss of BCS during the dry period (Chebel et al., 2018), and high levels of inflammation in the weeks before calving (Dubuc et al., 2010) have all been associated with reproductive tract diseases. Factors associated with changes in physiological and behavioural patterns in the weeks before calving show some promise in identifying cows at risk for disease. Also, major advancements in nutrition have informed the design of diets intended to modulate BCS of over-conditioned cows during lactation so that at the time of dry-off cows meet the targeted BCS (*e.g.*, Roche et al., 2013b).

Current understanding of lameness epidemiology and its causes in indoor housing systems point to the need to improve lying and standing behaviour, reduce BCS loss, use preventive hoof-trimming, barn hygiene and footbaths. There is a gap in literature reporting the epidemiology of lameness during the non-lactating period (i.e. two months before calving) and how lameness is associated with transition period disease. Studies covering this gap will likely provide recommendations for lameness management and prevention during the dry period.

In regard to pasture systems, research has been done on the effects of BCS on the transition period, such as reducing body condition towards the end of lactation by reducing diet's energy content (Roche et al., 2013a). However, the bulk of knowledge comes from Europe, where year-round grazing systems are rare, and from New Zealand where cows calve seasonally. In other parts of the globe dairy cows are kept on pasture year-round calving throughout the year. Studies in these systems are rare and often are carried out in single farms (*e.g.*, Giuliodori et al., 2013). Based on these studies it is difficult to say if transition period disease is actually a major issue in year-round grazing systems. Thus, there is need for descriptive work on transition period diseases in such systems. Understanding the occurrence of transition diseases in grazing systems will 1) help to assess the size of the issue and 2) indicate several factors associated with

transition diseases that may be further studied, resulting in recommending best practices to prevent disease.

The specific objectives of this thesis are:

1) To describe the prevalence and risk factors for transition period diseases in grazing dairy cows
2) To assess lameness during the dry period in indoor housed dairy cows, including epidemiology and associated factors
3) To measure the association between lameness during the dry period and transition period diseases

Chapter 2: Prevalence and risk factors for transition period diseases in grazing dairy cows in Brazil[3]

2.1 Introduction

One of the primary gaps in transition period of dairy cows' literature concerns to the lack of epidemiological research of transition period diseases on grazing dairy cows, and can be summarized in a simple question, is transition period diseases on grazing cattle a big problem? This chapter explores the epidemiology of transition period diseases in dairy cows under intensive grazing systems in southern Brazil.

There is a dearth of information on disease prevalence, disease between-herd variability and associated herd- and cow-level risk factors for dairy cattle disease in pasture-based dairies. Understanding the risk factors associated with transition period diseases in grazing herds may help to overcome some of these problems.

The prevalence of SCK in grazing dairies is not well described, with the exception of the recent work in New Zealand by Compton et al (2014) that reported a 24% herd prevalence of SCK (serum BHB ≥ 1.2mmol/L) in cows from 7 to 12 DIM, which is similar to SCK prevalence for indoor housed cows in the same period (see Duffield et al. 2009).

Metritis has been associated with decreased milk production, lower reproductive performance and early culling (Giuliodori et al., 2013). Several risk factors have been described for metritis, including dystocia, RP, and lower BCS (Dubuc et al., 2010). As with most

[3] A version of this chapter has been published: Daros, RR; Hötzel, MJ; Bran, JA; LeBlanc, SJ and von Keyserlingk, MAG. 2017. Prevalence and risk factors for transition period diseases in grazing dairy cows in Brazil. Prev. Vet. Med. 145: 16 – 22.

production diseases, few studies have been done on grazing cows. Bruun et al. (2002) reported that the incidence of metritis was lower for grazing cows than for housed cows.

RP has been associated with poor reproduction and lower milk production (Dubuc et al., 2011). The complex interactions between the stress response, the immune system and the occurrence of RP are not well understood (Beagley et al., 2010). To our knowledge no study has attempted to identify potential risk factors for RP in dairy cattle on pasture-based systems.

The objectives of this study were to measure the prevalence of the most common transition period diseases in intensively-managed grazing herds and to identify risk factors for SCK, metritis and RP, specifically focusing on management and environment-related factors.

2.2 Materials and methods

This study was carried out between February and October of 2015 in the western part of Santa Catarina State in Brazil, as part of a larger study that also focused on dairy cattle lameness and stakeholder views of dairy production in Brazil. All procedures were approved by the Ethics Committees on Research on Humans (Protocol # PP1237779, 2015) and Animals of the Universidade Federal de Santa Catarina (Protocol # PP00949, 2014) and by the UBC Animal Care Committee (Protocol # A15-0082).

2.2.1 Selection of participants

To capture representative variability in herd management practices and environmental conditions, and based on the time available to carry out the study, we set out to visit a minimum of 50 farms. The criteria for selection of dairy farms were herd size of approximately 40 to 100 cows and cows housed on pasture for at least 16 h/d. Potential participant farms were identified

by members of the research team via informants (i.e. people working in the dairy sector - public and private). To minimize potential selection bias, informants were only aware of the general aim of the study, i.e. to determine the prevalence of diseases on dairy farms in their region. After the farms were selected, the members of the team visited each farm, where they initially explained the study's general and specific methodologies, as well as their role in the study. Consent forms were read and explained to the farmers. We approached 61 farms initially, from which 53 farms provided their consent to participate in the study. For those who agreed to participate, a first visit was scheduled at a time that was convenient for the farmer.

2.2.2 Data collection

A three-step approach was used to collect the data for this study, including a semi-structured interview, inspections of the environment and examinations of the cows.

2.2.2.1 Questionnaire

The original questionnaire in Portuguese and its English version can be found online at https://doi.org/10.5683/SP2/Q4NL1C. The survey questionnaire was loaded onto smartphones to facilitate data collection and handling. The questions were verbally communicated to the farmer and their responses captured during a face-to-face interview that took place at the first visit. To initiate the conversation with the farmer, general information was collected in the first half of the interview, including location of the farm, size, number of cows and milk yield per cow. Questions regarding feeding management of milking and dry cows, dry period management, pre- and postpartum management, prevalence and incidence of diseases and health management were introduced in the second half of the interview. The interviews took from 1.5 to 3 h.

2.2.2.2 Environment inspection

All environment inspections were performed during the first and a subsequent visit (2 to 4 months apart); this allowed for one environmental inspection during the summer and a second during the winter months. During these visits we observed one milking and walked through the grazing paddocks used to house the lactating, dry, and close-up cows, the feeding barn and the holding areas. Data regarding floor surface cleanliness of the barn (0 = clean, 1 = dirty), access to water, access to shade, number of paddocks, types of grass, type of general milking management and time spent in holding areas waiting to be milked were recorded, as described below. All farms were intensively managed, as described by Balcão et al., (2016). Stocking density ranged from 2 to 3 cows/ha, cows were milked twice a day and had access to 2 to 3 fresh paddocks per day covered with specific grazing grasses such as *Cynodon dactylus* (var. Tifton 85 – Bermuda grass) and *Megathyrsus maximum* (var. BRS kurumi) during the summer months and *Avena sativa* (oat) and *Lolium perenne* (ryegrass) during the winter months. In all farms, cows were supplemented with corn silage and concentrate 1 to 3 times a day in a designated feeding area with headlocks. Cows were bred and calved throughout the year.

2.2.2.3 Cow examination

As visits were scheduled upon farmer availability, no randomization was used to select cows, i.e., all cows from 3 to 21 DIM were assessed at the visits. In order to assess at least 12 eligible cows per farm, we visited each farm between 2 and 6 times. Cows were not assessed more than once during the transition period and farmers were always present for cow inspections.

Cows were identified by farm and name or tag number and subjected to a number of measures. Cows were assigned a BCS while restrained in headlocks using a 0.25-increment

scale (Ferguson et al., 1994). A validated cow-side hand-held meter (Precision Xtra β-ketone, Abbott Diabetes Care) was used to measure BHB in whole blood (Iwersen et al., 2009) collected from the tail vein. Cows were considered to have SCK when BHB was ≥ 1.2mmol/L.

Metritis was assessed by visual/olfactory analysis of VD. After cleaning the vulva, the VD was collected by clean gloved hand covered in a lubricant solution. Discharge was scored: 1 = clear or slightly bloody or small flecks of pus, no foul smell; 2 = < 50% pus flecks, with foul smell; 3 = > 50% pus and foul smell; 4 = foul smell and red/brown watery discharge. Cows were considered healthy between 3 and 14 DIM if metritis score was 1, 2 or 3 and metritic if they had score of 4. Cows between 15 and 21 DIM were considered non-metritic when scored 1 and metritic if they had a score of 2, 3 or 4.

Clinical history of the last calving of each inspected cow was recorded as reported by the farmer: Dystocia - any type of calving assistance; RP - presence of retained fetal membranes > 24h; down cow – pathologically recumbent cow in the days around calving, likely attributable to hypocalcemia or injury; DA (including right and left displacements), milk production (L/d) on the day prior to the visit, as reported by the farmer.

2.2.3 Data handling

2.2.3.1 Cow-level data

All disease variables were coded as binary (yes=1 and no=0). Breed was classified as Holstein, Jersey, or crossbred (of which 90% were Holstein-Jersey crosses). BCS was classified as < 3, 3 to 3.5 or > 3.5. Parity was categorized as first, second, or third and greater lactation.

2.2.3.2 Herd-level data

The incidence of down cows was classified as low (<5%), medium (5-10%) or high (>10%). Access to water was classified as free (water trough in every paddock or the paddock gate was open to allow cows to access water elsewhere) or limited (cows housed in paddocks with no access to a water trough). Holding area cleanliness was used as a proxy for overall cleanliness and was measured before cows entered the holding areas for milking. These areas were considered dirty when a slurry layer covered the majority of the holding area on at least one of the visits and clean when no slurry or only a few dung piles were visible at both visits. Other factors asked about in the survey included use of a maternity pen (yes/no), if the calf was allowed to suckle the dam to obtain colostrum (yes/no), time that cow and calf were allowed together (categories: 0 to 12 h and > 12 h), and when cows joined the lactating herd after calving (categories: 0 to 12 h and > 12 h). For each disease, questions regarding disease management and treatment were asked.

2.2.4 Statistical analyses

Herd and disease data were summarized for each herd to produce within-herd disease prevalence estimates, for descriptive analyses. All data analyses were done using R language (R Core Team, 2016) and R packages lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2015).

A total of 662 cows from 53 farms were assessed for SCK, metritis and RP and binary coded for presence/absence of each disease. For categorical herd-level observations, explanatory variables with at least 8 observations per category were used to improve model fit, and variables with fewer than 8 observations were not considered for modeling.

Cow-level disease occurrence data were analyzed with separate multilevel logistic regression models for each disease. SCK, metritis and RP were modeled controlling for farm as a random effect. For the SCK model, 4 cows could not be assessed for BHB; thus 658 were used for this model. Seven cows were not assessed for metritis, resulting in 654 cows used in the metritis model. All 662 cows were used for modeling RP. Causal diagrams were drawn before analyses (not shown). Unconditional logistic regression models with herd as a random effect were used to screen variables one at a time, based on the logic in the causal diagrams. Variables with P-values ≤ 0.2 were considered as potential explanatory variables (see appendices B, C and D). Potential explanatory variables were assessed for collinearity and not included in further models when correlation was > 0.6; in these cases, the variable with most biological relevance was kept. This approach was chosen for simplicity; however, we acknowledge that we may have missed identifying some potential risk factors. Multivariable multilevel logistic regression models controlling for farm as a random effect were then built with all potential explanatory variables that passed the screening steps above, using 12 adaptive Gauss-Hermite quadrature points to estimate the variance of the random effect. The models were then reduced using backwards elimination. To control for confounding variables, after dropping any variable, changes in coefficients of the remaining predictors were inspected. Changes in coefficients greater than 30% were considered evidence of a confounder and these variables were kept in the model. Two-way interaction terms were tested among the variables in the final model using forward selection and dropped if their P-value > 0.05. Normality and homoscedasticity of residuals from higher level effects were assessed graphically.

To allow for the assessment of the between-herd variance the variation partition coefficients (VPC) were calculated using the latent response variable approach as suggested by Dohoo et al. (2012). VPC were calculated for final and null models of each disease.

2.3 Results

2.3.1 Descriptive statistics

Herds had an average of 37 lactating cows (min: 22, max: 67). The distribution of breeds was Holstein (65%), Jerseys (20%) and crossbred cows (15%); of BCS, < 3 (34%), 3 to 3.5 (50%) and > 3.5 (16%); and parity, first lactation (23%), second lactation (21%) and third or greater lactation (56%). Herd milk yield per lactation per cow (305d corrected) was on average 5,395 kg (min: 2,639, max: 8,259). On average we assessed 13 (±1.2, SD) cows per herd for transition period diseases. The overall prevalence of SCK and metritis, and incidence risk of RP, dystocia and down cow are presented in Table 2.1 and within-herd prevalence of SCK and metritis and incidence risk of RP, dystocia, down cow and displacement abomasum are presented in Table 2.2.

Table 2.1. Number of cases of transition period diseases and disorders in dairy cows in 53 grazing dairy herds in Southern Brazil in 2015

Condition	Total cows assessed	Positive cases	Overall proportion of cows affected (%)
Down cows	662	18	2.7
Dystocia	662	73	11.0
Retained placenta	661	92	13.9
Subclinical ketosis	658	136	20.7
Metritis	654	73	11.2

Table 2.2. Within-herd prevalence of subclinical ketosis and metritis and, incidence risk of other transition period disease from 53 grazing herds in Southern Brazil in 2015.

Disease & disorders	Mean herd prevalence or incidence risk (%)	Min	1st Q	Median	3rd Q	Max
Down cows[1]	6.4	0.0	0.0	3.3	8.3	34.2
Dystocia	13.8	0.0	0.0	8.3	20.0	87.5
Retained placenta	14.3	0.0	7.1	14.3	18.2	62.5
Subclinical ketosis	21.0	0.0	8.0	16.7	30.1	66.7
Metritis	11.7	0.0	0.0	8.3	16.7	50.0
Displaced abomasum[1, 2]	1.4	0.0	0.0	0.0	2.6	8.8

[1] Due to low incidence of down cows and displaced abomasum, farmer-reported annual incidence risk was used for calculations.
[2] Includes left and right DA.

2.3.2 Subclinical ketosis

Jerseys cows, older cows (≥ 3rd lactation), cows in a herd with >10% incidence of down cow or in a herd with limited access to water had greater odds of SCK (Table 2.3). Milk yield was not associated with SCK although this variable was kept in the model to control for potential confounding.

Table 2.3. Multilevel logistic regression model of risk factors for subclinical ketosis in 609[1] cows in 51[2] grazing dairy herds in Southern Brazil in 2015.

Risk Factor	Value	Estimate	SE	OR	OR 95% CI		P-value
					Lower	Upper	
Intercept		-3.11	0.60				
Breed	Holstein	reference					
	Crossbred	-0.04	0.35	1.04	0.53	2.06	0.90
	Jerseys	0.80	0.31	2.23	1.20	4.14	0.01
Body Condition	< 3.0	reference					
	3.0 - 3.5	0.48	0.26	1.61	0.96	2.69	0.07
	> 3.5	0.56	0.34	1.76	0.90	3.44	0.10
Parity	1st lactation	reference					
	2nd lactation	0.37	0.39	1.45	0.83	3.14	0.34
	≥ 3 lactation	1.07	0.33	2.91	1.52	5.54	0.001
DIM	Per 1 d increase	-0.04	0.02	0.96	0.92	1.00	0.08
Milk yield	Per 1 L/d increase	0.01	0.02	1.01	0.97	1.05	0.49
Access to water	Limited vs. free	0.61	0.26	1.85	1.11	3.09	0.02
Estimated incidence of down cows	<5%	reference					
	5-10%	0.43	0.34	1.53	0.79	2.97	0.20
	>10%	0.98	0.32	2.66	1.42	4.99	0.002

[1] We excluded 49 cows from this model; milk yield was not reported.
[2] Two herds and the cows observed on these farms were excluded for the model because the access to water variable was not assessed.

2.3.3 Metritis

Cows with BCS of 3 to 3.5 were at lower risk of having metritis compared to cows with BCS < 3. RP, DIM and being in a herd with a dirty holding area were associated with increased odds of a cow having metritis (Table 2.4).

Table 2.4. Multilevel logistic regression model for risk factors for metritis of 638[1] cows in 51 grazing dairy herds in Southern Brazil in 2015.

Risk Factor	Value	Estimate	SE	OR	OR 95% CI		P-value
					Lower	Upper	
Intercept		-4.37	0.60				
DIM	Per 1 d increase	0.11	0.03	1.12	1.05	1.19	<0.001
Body Condition	< 3.0	reference					
	3.0 - 3.5	-0.89	0.37	0.41	0.21	0.79	0.008
	> 3.5	-0.56	0.49	0.57	0.22	1.49	0.25
Retained placenta	Yes vs. no	2.97	0.35	19.46	9.89	38.29	<0.001
Holding area dirtiness	Dirty vs. clean	0.74	0.36	2.09	1.02	4.25	0.04

[1] Two herds and the cows observed on these farms were excluded for the model because the holding area cleanliness variable was not assessed.

2.3.4 Retained placenta

Older cows and cows with dystocia had higher odds of having RP. Jersey cows, farms that used a maternity pen and the amount of time cow and calf spent together (> 12 h compared to < 12h) were associated with a decreased risk of RP (Table 2.5).

Table 2.5. Multilevel logistic regression model of risk factors for retained placenta in 640[1] cows in 51 grazing dairy herds in Southern Brazil in 2015.

Risk Factor	Value	Estimate	SE	OR	OR 95% CI		P-value
					Lower	Upper	
Intercept		-1.98	0.33				
Breed	Holstein	reference					
	Crossbred	-0.57	0.37	1.77	0.86	3.62	0.12
	Jerseys	-2.11	0.60	0.12	0.04	0.40	<0.001
Parity	1st lactation	reference					
	2nd lactation	0.41	0.41	1.50	0.67	3.35	0.32
	≥ 3 lactation	0.86	0.34	2.36	1.21	4.62	0.01
Dystocia	Yes vs. no	1.09	0.31	2.96	1.61	5.43	<0.001
Period of time cow-calf together	More than 12h vs. up to 12h	-0.83	0.33	0.44	0.23	0.84	0.01
Maternity pen	Yes vs. no	-0.62	0.32	0.54	0.29	1.00	0.05

[1] Two herds and the cows assessed on these farms were excluded from the model because the use of maternity pen variable was not recorded.

2.3.5 Sources of variance

The summary of partitioning of variance at the cow and herd levels is presented in Table 2.6. The vast majority of the variance was at the cow level, especially for RP. VPC of final models showed even less between-herd variability as inclusion of predictors captured additional variation.

Table 2.6. Summary of proportion of variance of dependent variables explained at each hierarchical level in null models and final models of disease occurrence in grazing dairy herds in Southern Brazil in 2015.

Model[1]	Source of variation	Subclinical ketosis	Metritis	Retained placenta
Null	*Cow*	85%	89%	97%
Null	*Herd*	15%	11%	3%
Final	*Cow*	94%	90%	100%
Final	*Herd*	6%	10%	<1%

[1] Models were estimated by maximum likelihood. Variation partition coefficients were calculated using the latent response variable approach as suggested by Dohoo et al. (2012).

2.4 Discussion

This work is novel compared to other cross-sectional studies (e.g. Suthar et al., 2013; Compton et al., 2014) as we were able to collect more detailed data regarding management and environmental aspects of the farms visited because of the multiple visits made. Our study indicates that several management practices and environmental conditions are associated with common transition cow diseases in grazing dairy herds.

Overall, the prevalence of the transition period diseases found in our study is similar to studies done in other countries (e.g. Suthar et al., 2013). However, we found a higher prevalence of some transition period diseases than the few studies done in pasture-based systems. For example, Stevenson (2000) reported 5% incidence of ketosis and McDougall (2001) reported less than 2% RP and 4% assisted calving (dystocia). Although comparisons among studies are difficult due to differences in disease definitions and sampling strategies, our study suggests that

the incidences of RP, SCK, metritis, dystocia, and down cow are high in some grazing-based dairies.

Two recurrent risk factors for transition period diseases found in the literature are herd size (Robbins et al., 2016) and season (Suthar et al., 2013; Vanholder et al., 2015). In our study neither of these factors were associated with occurrence of SCK, metritis or RP. We attribute the lack of association between herd size and disease occurrence to the fact that we restricted herd size in our selection criteria, so there was little variation in number of cows among our study herds. Regarding season, some authors have suggested that during the winter months health is impaired and other type of feeds are used, thus increasing the likelihood of cows developing SCK and metritis (Bruun et al., 2002; Vanholder et al., 2015). During our study farmers did not change feed source (i.e. used the same concentrate and silage across seasons), however pasture plant species changed from primarily consisting of tropical grasses in the summer to temperate grasses in the winter.

As in any cross-sectional study, we do not intend to infer any causal relationship between the associated factors and the diseases studied. To minimize selection biases, we ensured that the participant farmers (and those who referred farms) were blind to the main objectives of the study. Nonetheless, we recognize that our study was based on a convenience sample and thus is not representative of the whole industry in the region. Furthermore, as multiple visits were required in order to collect sufficient cow-level data due to small herd sizes, we were able to re-check some of the information gathered and thus feel that we improved the quality of data captured.

2.4.1 Subclinical ketosis

Cows in herds that had limited access to water were more likely to have SCK. Lack of free access to water have been reported previously in several studies done in the same region (Costa et al., 2013; Balcão et al., 2016). Like ours, none of these studies measured water intake, but given that water management is associated with water intake (Pinheiro Machado Filho et al., 2004; Coimbra et al., 2012), cows with limited access to water may drink less water than needed. Reduced water intake is correlated with poor DMI, which may exacerbate negative energy balance; for example, in a study in a free-stall dairy, cows that had decreased DMI pre-partum were more likely to develop SCK postpartum (Goldhawk et al., 2009).

Only a few studies assessed SCK prevalence in different breeds. Swedish Friesian (Emanuelson et al., 1993) and crossbred Holstein × Jerseys (Ribeiro et al., 2013) have been reported to be at higher risk of SCK than Holsteins. In our study Jerseys had higher prevalence of SCK than Holsteins. Half of the Jersey cows were in mixed-breed herds, ruling out the hypothesis that Jerseys would have higher SCK prevalence simply because of herd management factors not assessed in our study. We also accounted for unmeasured sources of variance by including farm as a random effect. The prevalence of SCK in Jersey cows in mixed-breed herds was the same as the prevalence in Jersey-only herds (data not shown). A recent study also found Jerseys in intensively housed herds in Canada to have higher prevalence of SCK than Holsteins (Tatone et al., 2017). Further investigation is likely needed to understand if elevated BHB levels have the same negative effects across breeds.

Despite lower milk production in our population of grazing cows, compared to intensively housed dairy cows, the overall SCK prevalence was similar to that reported for non-grazing herds in USA (McArt et al., 2013) and in Europe (Suthar et al., 2013). The literature is

not clear whether milk production per se is associated with SCK (Raboisson et al., 2014), largely because SCK typically occurs very early in lactation. In the present study, milk yield was not associated with SCK at the cow-level.

Primiparous cows were at lower risk of having SCK compared to multiparous cows, a finding that is in accordance with other studies (Suthar et al., 2013; Vanholder et al., 2015). Furthermore multiparous cows are also more prone to other postpartum diseases, particularly those linked with SCK (Duffield, 2000; Suthar et al., 2013).

Some authors have suggested that high BCS during the dry period or at calving, combined with a greater loss of BCS post-partum are better predictors of SCK occurrence (Roche et al., 2009, 2013b). Unfortunately, we were not able to assess the loss of BCS over the transition period. We did observe a tendency for high BCS (>3.5) to be associated with SCK, which is likely explained by fatter cows at calving succumbing to greater body weight loss during the transition period (Roche et al., 2007).

The peak incidence of SCK in intensively managed herds in North America is normally observed during the first week of lactation, decreasing in the subsequent weeks (McArt et al., 2013), with some cases observed later in lactation (i.e. 4-6 weeks postpartum in pastured cows; Compton et al., 2014). Our results showed only a tendency for SCK prevalence to decrease from 3 to 21 DIM, thus we may have a higher prevalence of SCK in the second and third weeks postpartum, which could be related to different feeding regimes of grazing dairy cows compared to confined dairy cows.

2.4.2 Metritis

As expected, thinner cows and those diagnosed with RP were more likely to have metritis. These risk factors are consistent with several other studies that assessed different production systems (e.g. Bruun et al., 2002; Dubuc et al., 2010). The association of DIM with metritis may be an artifact of our disease definition (mild metritis between 15 and 21 DIM was included in the case definition). Holding area dirtiness was also associated with a higher chance of cows having metritis. We speculate that dirtiness of the holding area may be a proxy for general cleanliness of the facilities and perhaps hygiene at calving.

The prevalence of metritis reported here is likely underestimated, because we only examined each cow once during the transition period; thus, our underrepresentation of metritis likely biases our findings towards the null (Dohoo et al., 2012). This likely contributes to the fact that other cow and herd-level variables were not retained in the final models.

2.4.3 Retained placenta

Our results indicate that the use of a maternity pen and time spent together by cow and calf may impact RP occurrence. On the farms visited in this study, pre-partum cows were normally housed on a separate pasture paddock and thus extensively managed, which may have included lack of shade or insufficient water access. Providing access to a maternity pen may increase the likelihood of the cow having access to *ad libtum* feed, water, and shade. Increasing the time cows are away from the milking herd after calving might be a surrogate measure of farmer attention during the calving period. We speculate that farmers that allow more time for the cow to recover from calving are also more likely to closely monitor cows throughout the transition period, which could result in improved health.

In regards to the protective effect for RP that we found of having the cow and calf together after calving, most of the research looking at RP has focused on pre-partum factors that may influence the immune system and hormonal balance (Laven and Peters, 1996; Beagley et al., 2010). Thus it is difficult to explain the association between RP occurrence and presence of the calf, after placental detachment is understood to occur. However, to our knowledge no studies have investigated the presence of the calf in the first hours after calving and potential effects on RP. While suckling by the calf might increase oxytocin release, uterine contraction is not thought to be an important variable for occurrence of RP (Attupuram et al., 2016).

At the cow level, parity, breed, and dystocia were the main factors associated with occurrence of RP. Dystocia may cause trauma, which may impair cotyledon-caruncule separation (Beagley et al., 2010), further impairing placental detachment (Mordak and Anthony, 2015). It is worth noting that there was no standardization on the definition of dystocia across farms in our study so we considered any obstetrical assistance as dystocia. Also, with any self-reported farmer data there is always a risk of poor recall or other bias. However, we believe that given the small herd size, with few monthly calvings, the risk of poor recall regarding cases of dystocia was low.

Previous studies have varied with respect to identifying breed as a risk factor for RP (e.g. Bendixen et al., 1987; McDougall, 2001). In our study, Jerseys were less likely to have RP compared to Holstein cows. Several studies have reported higher incidence of RP in Jerseys, possibly because of their higher susceptibility for hypocalcemia (Curtis et al., 1983; Lean et al., 2006). Our work fails to substantiate this, which is similar to other large field trials that also failed to find breed associations between hypocalcemia and RP (Quiroz-Rocha et al., 2009; Chapinal et al., 2011).

2.5 Conclusion

Although the majority of the variation in disease occurrence was dependent on cow-level factors, the wide variation between herds visited and the several herd-level risk factors identified, provide evidence that environmental factors and management practices are associated with increased risk of disease, especially for SCK and metritis. These findings also identify opportunities for further research to help to prevent disease in grazing dairy cows.

Chapter 3: Lameness during the dry period of freestall housed dairy cows: epidemiology and associated factors[4]

3.1 Introduction

As I have argued previously, lameness is a painful condition (Chapinal et al., 2010) that affects 20 to 55% of indoor housed dairy cows in North America (von Keyserlingk et al., 2012; Solano et al., 2015). This malady has been shown to decrease milk production (Green et al., 2002; Archer et al., 2010), reproductive performance (Hernandez et al., 2005; Bicalho et al., 2007), and to increase involuntary culling (Booth et al., 2004). Though lameness can occur at any stage of a cow's life, the majority of work has focused on lactating cows. There is, however, no consensus as to when during lactation cows are at greatest risk for lameness. For example, Bicalho et al. (2007), found lameness to be more prevalent during the first weeks of calving, while Green et al. (2002) reported greater lameness prevalence in the 2 to 3 months after calving. Lastly, in an observational study including dry cows, Calderon and Cook (2011) found lameness to be highly prevalent during the dry period.

Most studies on lameness have reported lameness prevalence; thus, failing to provide information about when, and how many, new lameness cases arise or are cured. In the UK, where a few herds were regularly assessed for lameness (see Randall et al., 2015 for herd description), lameness incidence ranged from 1.4 to 7.4 cases of lameness per cow/year (Randall et al., 2018). Lameness cure is rarely reported; Lim et al. (2015) reported cure risk during lactation of 81%, with most (88%) curing from lameness within 45 d. Archer et al. (2010)

[4] A version of this chapter has been submitted for publication: Daros, RR; Eriksson, HK; Weary, DM and von Keyserlingk, MAG. Lameness during the dry period: epidemiology and associated factors.

reported that from cows gait scored in the months immediately before and after the dry period, 57% of cows remained lame, 18% recovered from lameness during the dry period and 16% developed new cases. There is a dearth of information about how lameness develops in non-lactating cows.

Lameness is usually caused by claw lesions (Murray et al., 1996; Tadich et al., 2010), which are commonly treated by hoof-trimming (see review by Potterton et al., 2012). However, the success of hoof-trimming treatment depends on the type of lesion (Miguel-Pacheco et al., 2017) and lameness duration (Thomas et al., 2016). Hoof-trimming can be used as a preventive strategy for lameness (Manske et al., 2002); thus, some industry organizations recommend hoof-trimming two months before calving (Dairy Farmers of Canada, 2009).

Other factors have been associated with the development of lameness. For example, thin cows are more likely to become lame compared to cows that maintain good body condition (Randall et al., 2015, 2018). Moreover, older cows, cows that produce more milk and cows with previous history of claw lesions are more likely to be lame (Green et al., 2002; Hirst et al., 2002; Lim et al., 2015; Randall et al., 2018).

The aims of this study were three-fold: 1) to measure the incidence and cure of lameness during the dry period, 2) to measure the association between lameness during the dry period and in the weeks after calving, and 3) to describe risk factors associated with onset, cure and chronic cases of lameness during the dry period.

3.2 Materials and methods

3.2.1 Farm enrollment criteria

Data collection was carried from May 2017 to January of 2018. Through a partnership with a local hoof-trimming company (AR-PE Hoof trimming Ltd., Abbotsford, Canada), commercial farms in the lower Fraser Valley, British Columbia, Canada were selected following several criteria: ≥ 160 lactating cows, freestall housed herd, individual records of cows, and willingness to participate in the study. In total 13 farms were contacted, from which 6 were used in the current study. Cows from participating farms were routinely hoof-trimmed by one of three certified hoof-trimmers from the same trimming company. In this prospective longitudinal project, cows were enrolled continuously; thus, herds were visited according to the data collection schedule, as described below. The project was approved by the Animal Care Committee at the University of British Columbia (UBC, protocol A15-0084).

Number of cows available for data analysis of the current study was set by the sample size required for the study described in Chapter 4 (see below); thus, no additional power calculations were undertaken for the specific objectives of the current study.

3.2.2 Farm description

Detailed description of the enrolled farms and farm management practices are provided in Table 3.1. Farm characteristics were recorded through a structured interview with the herd manager. Variables related to barn structure (e.g., flooring type and number of feed-space per pen) were assessed through an environmental inspection performed during the first farm visit. Selected farms milked on average 361 ± 137 (SD) cows with an average milk yield of 11866 ±

1579 kg of milk per lactation. Lactating cows were kept in freestall barns with either concrete flooring (n=5) or slatted floors (n=1). On one farm the far-off cows had outdoor access during the summer (from June to September). Standard practice on all farms was to dry off cows approximately 2 months before their expected calving date. Lactating herd lameness prevalence was assessed during the first farm visit. Lactating herd lameness prevalence was assessed during the first farm visit when cows were gait scored when exiting the milk parlor following the 5-point methodology described by Flower and Weary (2006). Cows were considered lame when gait score ≥ 3; all others were considered sound. Details on interobserver reliability are provided below.

Table 3.1 Farm characteristics and management for the 6 participating farms on the study of the epidemiology of lameness during the dry period. All farms were located in the Fraser Valley region, British Columbia – Canada.

Farm	A	B	C	D	E	F
Herd size[1]	185	510	540	310	330	290
Breed	Holstein	Holstein	Holstein	Holstein	Mixed[2]	Holstein
Milk production (kg/lactation)[3]	12718	12819	12942	10461	9134	12210
Lactating herd lameness prev. (%)	42	27	55	32	30	32
Cows per feed space[5]						
Dry pens	0.8	0.9	1.0	0.8	1.2	0.8
Lactating pens	1.0	1.0	0.9	1.0	1.1	1.3
Cows per lying space[6]						
Dry pens	1.0	0.7	0.8	0.8	1.0	0.9
Lactating pens	1.0	0.8	0.9	1.0	1.1	1.0
Days dry (mean)	58	64	59	62	63	57
Pen-changes dry-off to early lactation	5	5	5	5	5	6
Pen lay-out						
Far-off pens	freestall	freestall	freestall	freestall	freestall	freestall
Close-up pens	open pack	open pack	freestall	open pack	freestall	freestall
Pen flooring						
Far-off pens	concrete	concrete	concrete	concrete	concrete slats	rubber
Close-up pens	sawdust, concrete	sawdust, concrete	concrete	sawdust, concrete	concrete slats	rubber
Manure handling	scraper	scraper	flush	tractor	robot	scraper

[1] Sum of dry and lactating animals, pregnant heifers are not included.
[2] Holstein, Ayrshire, and Jersey and their crosses.
[3] Previous lactation (305 d corrected) kg of milk; data extracted from farm database for all enrolled cows.
[4] Dry cows fed every other day.
[5] One feed space is defined as either one head-lock, or 60 or 76 cm linear feed space for lactating and dry cows, respectively. Value represent average number of cows per lying space during the study period.
[6] One lying space is defined as either one freestall, or 11m^2 in open-pack pens. Value represent average number of cows per lying space during the study period.

3.2.3 Cow enrollment and data collection

A total of 465 parous cows with expected calving dates between July 21 and December 1 2017, were initially enrolled in the study. Data collection for each cow started at wk 9 before expected calving date. Cows were gait scored (details below) weekly until calving; cows that lost their ear tags (n = 2), died (n = 1), were sold (n = 3), calved too early (n = 3), or were reluctant to stand up because of severe lameness (n = 1) were excluded from the study, resulting in 455 cows with complete data sets during the dry period.

After calving, only a subset of cows (n=307) having 2 gait assessments within wks 2 or wk 3 were used for the analysis; cows with only 1 score were dropped. Within the cohort of 307 cows, 95 cows were followed until wk 8 or wk 9 after calving; however, this group of cows were part of another study assessing the first case of claw horn lesions which only included cows without previous history of claw horn lesions.

3.2.4 Hoof-trimming records

Lesion and treatment records were recorded using Hoof Supervisor System™ software (KS Dairy Consulting, Inc., Dresser, WI, USA) by the hoof-trimmer trained to use the Alberta Dairy Hoof Health Project's Lesion Severity Scoring Guide (www.dairyhoofhealth.info/Lesion-Severity-Guide-v0.7.pdf). Individual cow ear-tag numbers, presence of lesion, type of lesion, and date of trimming were retrieved from each farm from a data base containing all trimming records for the year 2017.

Hoof-trimmings performed from d 100 before calving to the day of calving were retrieved for each enrolled cow. Hoof-trimming data were summarized by cow per trimming event; a cow was considered affected when at least one lesion was recorded. Lesions types were

categorized as non-infectious lesions – that included severe sole hemorrhages, sole and toe ulcers, white line disease, periopole ulcers and thin soles – or infectious lesions – that included digital and interditgital dermatitis, and foot rot. Trimmings records were retrospectively split between periods, before enrollment (from d 100 before calving to enrollment) and after enrollment. If the cow was trimmed more than once before enrollment, the data from the trimming closest to enrollment date was used. In cases where there were multiple trimming records per cow after enrollment only the most severe lesion score was retained.

With the exception of one farm, all farms reported that cows were trimmed shortly before dry-off but during our observations it became apparent that some cows were not trimmed before dry-off. Thus, cows that were not hoof trimmed between d 100 before and calving were assigned as not trimmed. Hoof-trimming occurred on average 21 d before dry-off (25^{th} percentile = 9 d, 75^{th} percentile 49 d before dry-off).

3.2.5 Gait scoring and lameness definition

On each visit, gait scoring was performed by one of two trained observers, blind to the hoof-trimming records. A detailed description of training and interobserver reliability between the observers is reported in a companion paper by Eriksson et al. (2019). In brief, both observers scored cows on all farms. Interobserver agreement was calculated using the quadratic weighted kappa (Cohen, 1968). Resulting kappa values were 0.84 for scores from video recordings and 0.57 and 0.55 from live scoring performed at the beginning and after the end of the data collection period, respectively. Systemic bias between observers was measured using the bias index (Byrt et al., 1993) resulting in values very close to zero, indicating no systemic bias between the observers.

Cows were individually walked in the pen by the observer, and gait scored from behind using the 5-point scale described by Flower and Weary (2006). A cow was considered lame if she had 2 consecutive gait scores of 3 or had one gait score ≥ 4 (validated by Eriksson et al., 2019). Conversely, a cow was considered sound (or cured from lameness) if she had 2 consecutive gait scores ≤ 2. From these criteria we assigned a weekly lameness status for each cow. Based on the sequence of lameness status during the dry period each cow was categorized as follows: *chronically lame* (cows lame for the whole dry period), *always sound* (cows sound for the whole dry period), *became lame* (cows initially categorized as sound but becoming lame at any time during the dry period) or *cured* (cows that was initially lame and became sound at any time during the dry period). Cows that became lame, were further classified into *remained lame* (cow was lame on the last assessment before calving) or *recovered* (cows were sound on the last assessment before calving). Likewise, cows that cured were further categorized as *remained cured* (cow was cured on the last assessment before calving) or *reoccurred* (cow was lame in the last assessment before calving) (Figure 3.1). When severe lameness was identified it was reported immediately to the farm personnel, but we do not know if any treatment was provided.

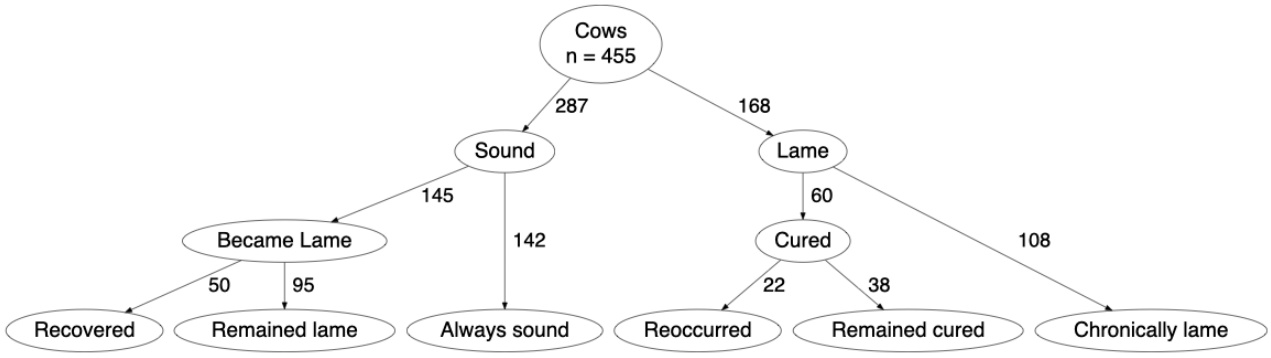

Figure 3.1 Diagram of lameness status during the dry period of dairy cows.
Numbers represent the total of cows in each lameness status category. From top to bottom: Total cows with complete data for the dry period (n=455); lameness status at first assessment; first lameness status change; final category based on last status assed in the week of calving.

3.2.6 Other cow variables

BCS (scale: 1 = thin to 5 = fat) was assessed using 0.5 increments following Ferguson et al. (1994). BCS was assessed fortnightly, starting 1 wk after enrollment. BCS was categorized as: < 3.0 = thin, 3.0 to 3.5 = good, and > 3.5 = fat. BCS was assessed by 4 jointly trained observers. Interobserver agreement between observers was calculated using the intra-class correlation (ICC), through *two-way* and *agreement* methods (Hallgren, 2012). ICC value of 1 indicates excellent agreement and value of 0 indicates agreement no better than chance; the calculated ICC for the 4 observers was 0.81 (95% CI: 0.73 – 0.87) indicating good to excellent agreement (see Cicchetti, 1994).

Parity at enrollment was categorized as either primiparous or multiparous. Previous lactation milk production for each cow (kg of milk per lactation - 305d corrected) was retrieved from the farms' database. Individual milk production was further centered and scaled; values used in the models represent standard deviation (SD) from the mean milk production of enrolled cows.

3.2.7 Statistical analyses

Statistical analyses were performed in R 3.5.0 via RStudio interface (RStudio Team, 2016; R Core Team, 2019). The list of R statistical packages used, full statistical analyses code and output, and data used for the analysis are available online at https://doi.org/10.5683/SP2/Q4NL1C.

Lameness prevalence at wk -8 (± 1 wk) in relation to the calving date was calculated as the number of cows that were classified as lame (using criteria defined above) in the first assessment divided by the number of cows enrolled in the study. Lameness prevalence at calving

was calculated as the number of cows classified as lame at the last gait score before calving divided by the total cows enrolled in the study. Similarly, the prevalence of post-calving phases was calculated based on the lameness status assigned at approximately wk 2 (± 1 wk) and wk 8 (± 1 wk) divided by the respective number of cows assessed in the same period.

Lameness incidence risk (i.e. cumulative incidence) during the dry period was calculated as the number of cows that became lame during the dry period divided by the number of sound cows at the beginning of the study period. Lameness cure risk during the dry period was calculated as the number of lame cows that cured lameness during the dry period divided by the number of lame cows at the beginning of the study period. Further classification was done for cows that became lame and cured of lameness sometime during the study period (see Figure 3.1). As such, incidence risk for the categories *recovered* and *reoccurred* were derived from the number of cows that became lame and cured, respectively.

Reporting rates allows for comparisons across studies as this measure considers when animals develop multiple cases of a given condition through the study period; thus, we also calculated lameness incidence rate and cure rate. Rates were calculated as the number of new cases of lameness or cure cases divided by the total number of weeks at risk, multiplied by 100. Number of weeks at risk per cow was calculated using the exact method as suggested by Dohoo et al., (2012).

A series of multilevel models (described below) were built to address the aims of our study. For all models, multicollinearity among variables was assessed though variation inflation factor; values > 3 were considered multicollinear except for interaction terms and their main effects (as they inherently would have a degree of structural collinearity). Variation inflation factor for main effects were never greater than the set threshold and interaction terms were never

> 4. Models were tested for goodness of fit using the Hosmer-Lemeshow test (number of groups = 10; Hosmer and Lemeshow, 1980). The lowest Hosmer-Lemeshow chi-square test p-value was 0.5, indicating that models fit the observed data. Selection of predictors to be included in the models was based on causal diagrams (i.e. no variable reduction procedure was used for the main effects). Sometimes a categorical variable had too few observations per level (≤ 5), impairing the estimation of its fixed effect standard error; in these cases, observations were either combined within another category or removed from the data and the model was re-fitted – details for each of these cases are provided below. Biologically plausible interactions were included in the model using manual forward selection and were only kept in the model if $P < 0.05$. If including the interaction term caused failure in model convergence the interaction and its main effects were explored in a separate model. The list of potential predictors available to be included in the models and their descriptive statistics are presented in Table 3.2. It was not the aim of the current study to evaluate herd-level variables thus herd-level variables were not included in the models. We have specifically chosen to include (where applicable) the random intercept of farm instead of including farm as fixed effect (n = 6 for most models) because 1) we did not have any a priori hypothesis about farm differences and, 2) we still wanted to control for some of the variation in the data arising from unmeasured farm level variables.

Table 3.2 List of potential predictors to be included in the models for assessing risk factors for the onset, cure and chronic lameness in 455 cows from 6 dairy farms in the Fraser Valley region, British Columbia – Canada.

Variable			Number of cows	Proportion or mean (± SD)
Name	Level	Type		
Parity at enrollment	*Primiparous*	Categorical	*162*	36 %
	Multiparous		*293*	74 %
BCS at enrollment	*< 3.0*	Categorical	*35*	8 %
	3.0 – 3.5		*302*	76 %
	> 3.5		*118*	26 %
Milk yield (kg/lactation)		Continuous	454	11860 (± 2573)
Hoof-trimming	*Before enrollment*	Categorical	*205*	45 %
	After enrollment		*44*	10 %
	Not trimmed		*206*	45 %
Hoof lesions	*Non-infectious lesions*	Categorical	*26*	13%
	Infectious lesions		*26*	13%
	No lesion		*153*	74%
Days elapsed from trimming to enrollment		Continuous	*205*	20 (± 12.3)

To measure the association between lameness diagnosed during the dry period with lameness at wk 2 and wk 8 post-calving, multilevel logistic regressions were fitted using lameness (binary: sound = 0 and lame = 1) at wk 2 and wk 8 as the outcome. Predictors included the main effect of lameness status at calving (binary: sound = 0 and lame = 1) and parity at enrollment.

Multilevel logistic regressions with farm as a random effect were fitted to assess risk factors for: 1) lameness onset (binary: *always sound* = 0, *remained lame* = 1); 2) lameness cure (binary: *chronically lame* = 0, *remained cured* = 1) and 3) chronic lameness (binary: *always sound* = 0, *chronically lame* = 1) during the dry period. Cows categorized as *recovered* or *reoccurred* were not used for these models as we predicted that they would add noise to the data, given that they both cured and became lame during the dry period. Detailed description of the models used to assess risk factors for the onset, cure and chronic lameness are provided below.

Normality of random effects was assessed graphically; no major deviations were observed by inspections of quantile-quantile plots.

3.2.7.1 Models: Lameness onset

Two different models were fitted to assess risk factors for lameness onset. The first included a subset of 237 cows (all *always sound*, and all *remained lame* cows), and set out to assess the association between hoof-trimming before enrollment (yes *vs* no) and the onset of lameness; this model also included, parity, milk production, BCS and the interaction between hoof-trimming before enrollment and parity. The second model included only cows that were hoof-trimmed before enrollment and assessed the association between having a hoof lesion, or not, before enrollment and the onset of lameness. From the subset of cows available for this latter model, only 2 cows had non-infectious lesions; hence, we combined different types of lesions into one category, encompassing cows that had either non-infectious or infectious lesions. Given that only 5 cows had BCS < 3 we removed these cows from the subset and fitted the model using data from 99 cows. This model included parity, hoof lesion, milk production, and number of days elapsed since trimming as predictors in the model.

3.2.7.2 Models: Lameness cure

Only one model was fitted to assess risk factors for lameness cure during the dry period. Data used for this model included 146 cows (all *chronically lame* and all *remained cured* cows). This model included parity, milk production, BCS and hoof-trimming before enrollment as predictors. We were not able to fit a second model to assess the association between type of lesion before enrollment and lameness cure, due to limited cow numbers (n = 76 cows) which prohibited model convergence for BCS and lesion type.

3.2.7.3 Models: Chronic lameness

Two models were fitted to assess risk factors for chronic lameness. The first model included data from 250 cows (all *always sound* and all *chronically lame* cows) and set out to assess the association between hoof-trimming before enrollment (yes *vs* no) and chronic lameness. This model also included, parity, milk production, BCS and the interaction between hoof-trimming before enrollment and parity. The second model included only cows that were hoof-trimmed before enrollment (n=120); however, due to the low number of thin cows (BCS < 3) we were required to drop these animals leaving a final dataset of 110 cows. Predictors included in this model were parity, type of lesion before enrollment, milk production and days elapsed from hoof-trimming to enrollment.

3.3 Results

3.3.1 Epidemiology of lameness and hoof lesion prevalence during the dry period

The prevalence of lameness and severe lameness at dry-off (~ wk -8; Figure 3.2), the wk of calving (Figure 3.2), in the early post-calving period (wk 2), and around peak lactation (wk 8) are presented in Table 3.3.

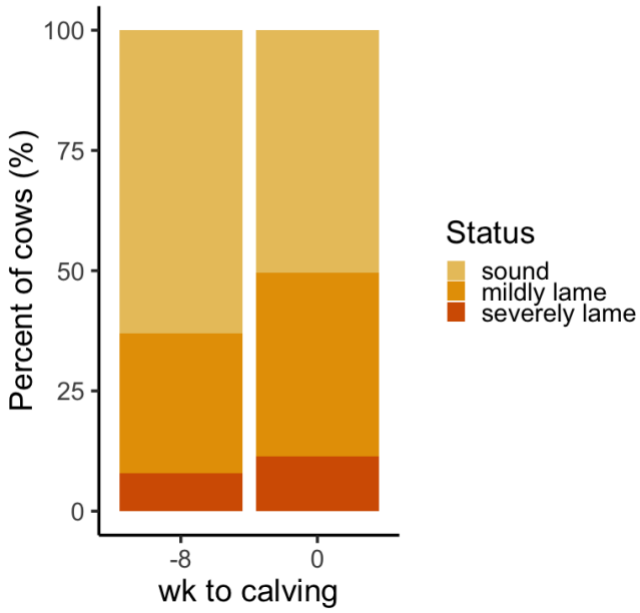

Figure 3.2 Prevalence of lameness at dry-off and on the week of calving by lameness severity.

Table 3.3 Parity and total prevalence of lameness at dry-off (week-8), at calving (week 0), early post-calving (week 2) and around peak lactation (week 8) of 6 dairy farms in the Fraser Valley in British Columbia – Canada.

Period	Condition category	Primiparous[1]	Multiparous	Total
Wk -8	Lameness (%)	9 %	28 %	37 %
	Severe lameness (%)	1 %	7 %	8 %
Wk 0	Lameness (%)	12 %	38 %	50 %
	Severe lameness (%)	3 %	8 %	11 %
Wk 2	Lameness (%)	12 %	34 %	46 %
	Severe lameness (%)	2 %	7 %	9 %
Wk 8	Lameness (%)	16 %	21 %	37 %
	Severe lameness (%)	4 %	3 %	7 %

[1] Parity at the time of enrollment.

The prevalence of hoof lesions in the 100 days before calving and the proportion of cows trimmed before and after enrollment are presented in Table 3.4.

Table 3.4 Prevalence of hoof lesions from day 100 before calving to the day of calving by period (before and after enrollment) and parity for 205 dairy cows in 6 dairy farms in the Fraser Valley region in British Columbia – Canada.

		Parity		
Hoof-trimming period	Lesion type	Primiparous[4]	Multiparous	Prevalence
Before enrollment	Non-infectious [2]	2 %	8 %	10 %
	Infectious [3]	3 %	7 %	10 %
	No lesions	95 %	85 %	80 %
After enrollment	Non-infectious [4]	0 %	2 %	2 %
	Infectious [3]	3 %	5 %	8 %
	No lesions	97 %	93 %	90 %

[1] Hoof trimmed performed by professional hoof trimmer. Lesion assessment based on Alberta hoof lesion atlas (available at www.dairyhoofhealth.info/Lesion-Severity-Guide-v0.7.pdf).
[2] Non-infectious include cows with severe sole hemorrhages, sole and toe ulcers, white line disease and thin soles.
[3] Infectious lesions include cows with digital dermatitis, interdigital dermatitis and foot rot.
[4] Parity at the time of enrollment.

The incidence risk for each lameness status category and average number of weeks lame per lameness category are described in Table 3.5.

Table 3.5 Epidemiological descriptors of lameness categories and median number of weeks lame during the dry period of 455 dairy cows from 6 dairy farms in the Fraser Valley region, British Columbia – Canada.

Lameness category[1]	Incidence risk (%)	proportion per group (%)[2]	Median weeks lame	1st quartile (Q1)	3rd quartile (Q3)
Starting as sound					
Became lame	50	-	4	2	5
Recovered	*34*	11	*2*	*2*	*4*
Remained lame	*66*	21	*4*	*2.5*	*6*
Always sound	50	31	-	-	-
Starting as lame					
Cured	36	-	5	3.75	7
Reoccurred	*37*	5	*6*	*4.25*	*7*
Remained cured	*63*	8	*5*	*3*	*7*
Chronically lame	64	24	-	-	-

[1] Values and categories indented to the right represent sub-categories.
[2] Proportion of animals in each lameness category in the week of calving in relation to the number of enrolled cows (n = 455).

Lameness incidence rate from enrollment to calving was 8.2 cases/100 cows/wk and the cure rate from enrollment to calving was 7.1 cases/100 cows/wk. The majority of cows that became lame had 1 new case of lameness during the dry period and only 11 cows had 2 new cases of lameness during the same period. Cows with multiple new cases of lameness were all lame at calving. Likewise, most of the cows that cured had 1 cure case during the dry period,

while only 4 cows had 2 cure cases during the same period. Cows with multiple cure cases of lameness were all sound at calving. The distribution of new cases of lameness and cure cases across the dry period are presented in Figure 3.3.

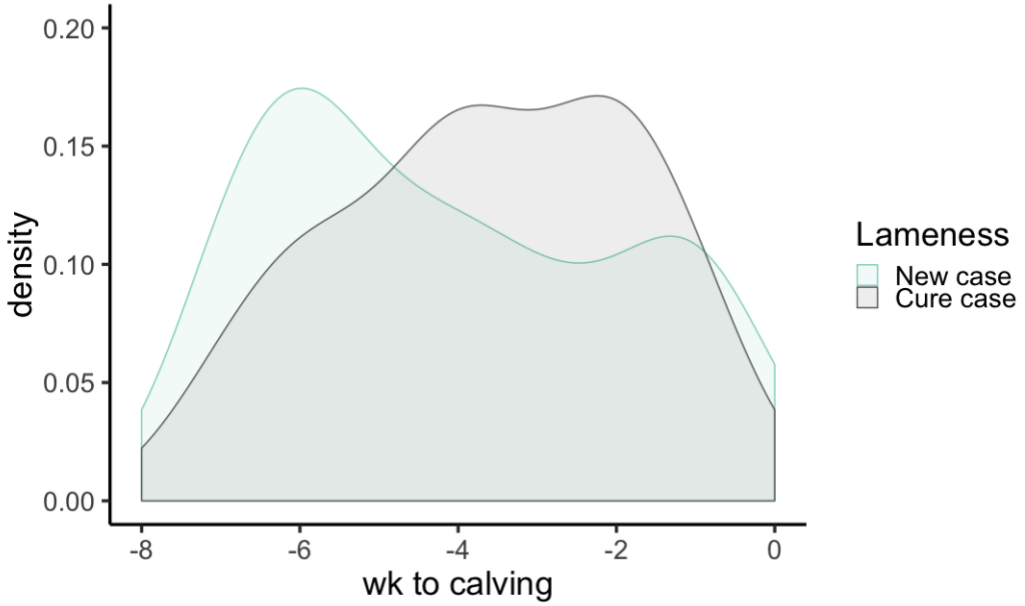

Figure 3.3 The distribution of new cases and cure cases of lameness during the dry period. Density was calculated based on the number of cases per wk divided by the total number of cases during the period dry period for respective category.

3.3.2 Association between lameness before and after calving

Cows that were lame immediately before calving had increased odds of being lame in wk 2 (OR = 37.0; 95% CI: 18.8 to 78.4; $P < 0.01$) and wk 8 (OR = 4.5; 95% CI: 1.8 to 12.0; $P < 0.01$) after calving. Parity was not associated with odds of being lame in either of the post-calving phases.

3.3.3 Risk factors for lameness onset

The interaction between parity and hoof-trimming was associated with the onset of lameness (see Table 3.6 and Figure 3.4). Multiparous cows had higher odds of becoming lame if

they were trimmed before enrollment while primiparous cows had lower odds of becoming lame if they were trimmed before enrollment. Milk production and BCS at enrollment were not associated with the odds of becoming lame.

Table 3.6 Multilevel logistic regression model of risk factors for becoming lame during the dry period in 237 cows in 6 freestall dairy herds in British Columbia – Canada.

					OR 95% CI		
Predictor	Level	Estimate	SE	OR	2.5%	97.5%	*P*
Intercept	-	-1.22	0.5	-	-	-	-
Milk production[1]	-	-0.02	0.2	0.98	0.68	1.39	0.93
Parity	Primiparous	Ref.	-	-	-	-	-
	Multiparous	0.73	0.4	2.09	0.90	5.00	0.09
BCS	> 3.5	Ref.	-	-	-	-	-
	3.0 to 3.5	0.46	0.4	1.58	0.79	3.22	0.20
	< 3.0	0.84	0.7	2.30	0.56	9.90	0.25
Hoof trimming before enrollment	no	Ref.	-	-	-	-	-
	yes	-0.89	0.5	0.41	0.14	1.15	0.10
Parity * hoof trimming	Primiparous * no	Ref.	-	-	-	-	-
	Multiparous * yes	1.54	0.6	4.67	1.35	17.25	0.02

[1] Scaled variable.

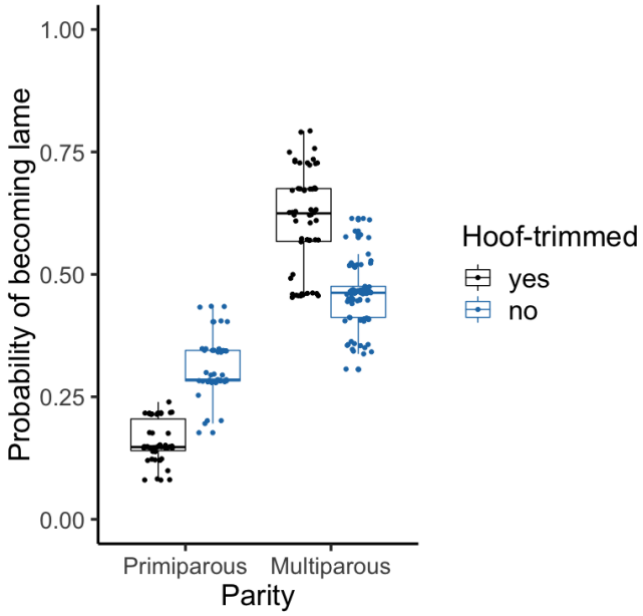

Figure 3.4 Estimated probabilities for each cow (single dots) of becoming lame from the interaction between the predictors hoof-trimming before enrollment and parity.

In the second model, which included only cows hoof-trimmed before enrollment, only parity was associated with the onset of lameness. Multiparous cows had 10 times the odds (95%

CI: 3.7 to 40.2; $P < 0.01$) of becoming and remaining lame during the dry period compared to primiparous cows. Having a hoof lesion (OR = 2.0; 95% CI: 0.5 to 10.4*;* $P = 0.38$), days elapsed from hoof-trimming to enrollment (OR = 1.0; 95% CI: 1.0 to 1.1; $P = 0.85$) and previous lactation milk production (OR = 0.7; 95% CI: 0.4 to 1.3; $P = 0.27$) were not associated with the odds of becoming and remaining lame during the dry period.

3.3.4 Risk factors for lameness cure

Parity and BCS were associated with the odds of curing lameness during the dry period (Table 3.7). Multiparous cows had lower odds of curing lameness compared to primiparous cows, and thin cows (BCS < 3.0) had lower odds of curing lameness compared to cows in good condition (BCS 3.0 to 3.5). Over condition (BCS >3.5) was not associated with changes in odds of curing lameness.

Table 3.7 Multilevel logistic regression model of risk factors for curing lameness during the dry period in 146 cows in 6 freestall dairy herds in British Columbia – Canada.

					OR 95% CI		
Predictor	Level	Estimate	SE	OR	2.5%	97.5%	*P*
Intercept	-	-0.12	0.6	-	-	-	-
Milk production[1]	-	0.03	0.2	1.03	0.64	1.70	0.89
Parity	Primiparous	Ref.	-	-	-	-	-
	Multiparous	-1.07	0.5	0.34	0.11	0.95	0.04
BCS	3.0 – 3.5	Ref.	-	-	-	-	-
	> 3.5	-0.11	0.5	0.90	0.31	2.52	0.84
	< 3.0	-1.85	1.1	0.16	0.01	0.95	0.09[2]
Hoof trimming before	no	Ref.	-	-	-	-	-
enrollment	yes	0.17	0.5	1.19	0.48	3.00	0.71

[1] Scaled variable.
[2] Discrepancy between *P* and 95% CI is because of different methods for estimating P (Wald method) and 95% CI (profile likelihood method).

3.3.5 Risk factors for chronic lameness

In the first model that included all cows regardless of whether they were trimmed before enrollment, the interaction between parity and hoof-trimming before enrollment was associated with the odds of being chronically lame. The estimates, confidence intervals and p-values for all

variables included in the model are presented on Table 3.8. The interaction follows the same pattern described for the onset of lameness; multiparous cows had higher odds of being chronically lame if they were trimmed before enrollment while primiparous cows had lower odds of being chronically lame if they were trimmed before enrollment (see Figure 3.5). Thin cows (BCS < 3) had increased odds of being chronically lame compared to cows in good condition (BCS 3.0 to 3.5) while over conditioned cows did not differ from cows in good condition.

Table 3.8 Multilevel logistic regression model of risk factors for chronic lameness during the dry period in 250 cows in 6 freestall dairy herds in British Columbia – Canada.

					OR 95% CI		
Predictor	Level	Estimate	SE	OR	2.5%	97.5%	*P*
Intercept	-	-1.29	0.6	-	-	-	-
Milk production[1]	-	-0.28	0.2	0.76	0.51	1.11	0.16
Parity	Primiparous	Ref.	-	-	-	-	-
	Multiparous	1.16	0.5	3.19	1.26	8.50	0.02
BCS	3.0 – 3.5	Ref.	-	-	-	-	-
	> 3.5	-0.41	0.4	0.66	0.31	1.39	0.29
	< 3.0	1.25	0.6	3.50	1.14	12.37	0.04
Hoof trimming before enrollment	no	Ref.	-	-	-	-	-
	yes	-0.80	0.6	0.45	0.14	1.33	0.15
Parity * hoof trimming	Primiparous * no	Ref.	-	-	-	-	-
	Multiparous * yes	1.61	0.7	5.02	1.35	19.44	0.02

[1] Scaled variable.

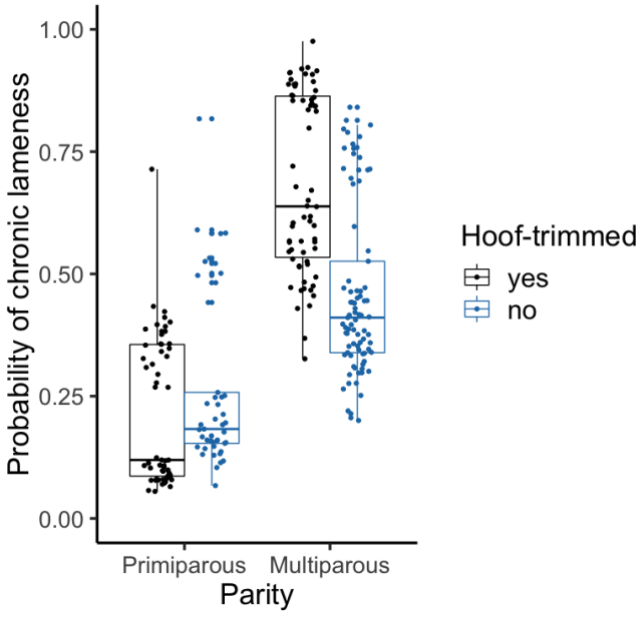

Figure 3.5 Estimated probabilities for each cow (single dots) of chronic lameness from the interaction between the predictors hoof-trimming before enrollment and parity.

In the second model, that included only cows that were hoof-trimmed before enrollment, parity and lesion type were associated with the odds of chronic lameness. Multiparous cows had increased odds for chronic lameness (OR = 10.8; 95% CI: 3.4 to 44.4; $P < 0.01$) compared to primiparous cows. Cows diagnosed with non-infectious hoof lesions before enrollment also had increased odds for chronic lameness (OR = 38.9; 95% CI: 5.8 to 822; $P < 0.01$) compared with cows that were not diagnosed with any hoof lesion in the same period. Infectious lesion (OR = 2.9; 95% CI: 0.6 to 16.6; $P = 0.22$), days elapsed from hoof-trimming to enrollment (OR = 1.0; 95% CI: 0.9 to 1.0; $P = 0.15$), and estimated lactational milk production (OR = 0.7; 95% CI: 0.4 to 1.3; $P = 0.50$) were not associated with the odds of chronic lameness during the dry period.

3.4 Discussion

Our study is the first to describe incidence and cure rates and risk of lameness during the dry period. In this study we report both risk and rate to provide a comprehensive level of detail of the lameness new cases and cure cases. While risk provides the exact proportion of cows becoming lame or curing lame during the dry period, rates provide a measurement that can be generalized as this measurement is given in animal-time, allowing for calculations of how the incidence would be, had we conducted the study for longer (e.g. 1 year). An extrapolation of our data suggests a yearly incidence rate of 4.2 cases/cow/year. In a recent study, using weekly gait scoring in a research dairy herd, Randall et al. (2018) report lactational lameness incidence 7.4 cases/cow/year. In other studies, lactational incidence rates of lameness ranged from 0.1 to 1.7 cases/cow/year in a study of 37 dairy farms (Clarkson et al., 1996) and an average of 0.7 cases/cow/year in another study of 5 dairy farms (Green et al., 2002). It is worth noting that these

two studies did not assess lameness as frequently as we did. Following our lameness assessment schedule and definition we were able to detect short cases of lameness. Moreover, we did not enroll late gestation heifers, a group of animals with a lower lameness prevalence compared to parous cow (Calderon and Cook, 2011). Given our case definition, and the inclusion of only primiparous and older cows, it is not surprising that we found a higher incidence of lameness.

Few studies have reported cure from lameness during the dry period. For instance, Archer et al. (2010) reported that lameness cure risk (i.e. proportion of cows that were lame before dry-off and were sound after calving) was 18%. Our results show that 36% of cows cured lameness during the dry period, however, from these 37% reoccurred in the weeks before calving. Lim et al. (2015) reported a lactational cure risk of 81% but these authors followed cows for a much longer period of time; 88% of cure cases occurred within 45 d after the lameness episode. Perhaps if we had followed all enrolled cows beyond the dry period a higher proportion of lameness cases would have had time to recover. Nonetheless our results suggest that the dry period may not be long enough for some cows to recover from lameness.

A total of 11% of the cows developed short cases of lameness; these cows were lame for < 4 weeks during the dry period. In a companion study (Eriksson et al.), we suggest that using two consecutives gait scores to classify lameness cases can decrease misclassification, while still detecting lameness cases of short duration. Little is known about the effect of short lameness cases; however, it is likely that even short cases of duration increase the chances of future lameness cases (Randall et al., 2018).

We graphically assessed the distribution of new lameness cases and lameness cure cases during the dry period (see Figure 3.3). A lower proportion of cases at the beginning of the dry period and around calving were expected, given that our lameness case definition required two

consecutive scores. Although we observed a decrease in the proportion of new cases of lameness over the dry period, we also observed a higher proportion of cure cases later on during the same period. We speculate that these patterns may indicate that there is a shift in the risk of becoming lame throughout the dry period. Future studies may further investigate this idea by looking at risk factors for the development and cure cases of lameness in the far-off and close-up periods separately.

In contrast to the low prevalence of lameness around dry-off reported by Foditsch et al. (2016), our results show a high prevalence of lameness around dry-off and calving in line with those described by Archer et al. (2010) that reported lameness prevalence of 66 and 73% in the month before dry-off and the month after calving, respectively. Discrepancies in lameness prevalence may be due to herd selection criteria and gait scoring methodology used.

In the current study, cows that were lame in the wk before calving were also more likely to be lame around wk 2 and wk 8 after calving. This result suggests that a large proportion of lameness during the first months of lactation may have been carried over from the dry period, likely contributing to the high prevalence of lameness in early lactation (Green et al., 2002; Bicalho et al., 2007).

Despite the fact that previous lameness events are known to be a major risk factor for future lameness events (Randall et al., 2018), we were unable to include this information in our models given that our work took place on commercial dairy farms that do not routinely collect lameness events. We encourage the reader to consider this when interpreting our findings. In the current study, hoof-trimming before the enrolment period was associated with decreased odds of becoming lame and chronic lameness for primiparous but not multiparous cows. Randomized studies assessing the effectiveness of hoof-trimming on future lameness occurrence (Manske et

al., 2002) or lameness cure shortly after trimming (Thomas et al., 2015, 2016) did not report this interaction. Cows with a previous history of claw lesions and lameness are more likely to develop subsequent cases of lameness (Hirst et al., 2002; Randall et al., 2018) possibly explaining why older cows are more likely to be lame and have non-infectious hoof lesions (Amory et al., 2008; Foditsch et al., 2016). We further speculate that farmers could be selecting (consciously or not) multiparous cows for trimming that had a history of claw lesions and lameness. Conversely, primiparous cows may have not yet developed lameness (or claw lesions), and thus were better able to benefit from the preventive effect of hoof-trimming. Hoof-trimming treatment outcome depends on when trimming is performed in relation to the onset of lameness (Leach et al., 2012; Thomas et al., 2016); therefore if multiparous cows have been previously lame, or chronically lame, it is less likely that hoof-trimming would be effective for them. Further research should investigate the effect of preventive trimming before dry-off for cows with and without previous history of lameness and hoof lesions.

We found that cows with non-infectious lesions were more likely to be chronically lame during the dry period compared with cows with no lesions. Non-infectious lesions have been associated with the formation of bone protrusions (i.e. osteomas) in caudal region of the distal phalanx (Newsome et al., 2016); their presence may explain why cows with previous non-infectious lesions would be at increased risk of developing subsequent cases of lesions and lameness (Hirst et al., 2002; Foditsch et al., 2016).

Body condition at dry-off has been positively associated with digital cushion thickness (Machado et al., 2011) and is thought to reduce the risk of the development of non-infectious lesions (see review: Bicalho and Oikonomou, 2013). Accordingly, several studies have shown that having low BCS is associated with the development of lameness (Lim et al., 2015; Randall

et al., 2015). As expected, we found that low body condition around dry-off was associated with higher odds of chronic lameness and lower odds of curing from being lame during the dry period. However, low body condition was not associated with odds of becoming lame. Perhaps, the low number of thin cows available may have made it difficult to detect such effect, given that body condition only contributes slightly to the risk of lameness development (Randall et al., 2018).

Previous lactation milk production was not associated with lameness onset, lameness cure or chronic lameness. Because our sample size used to measure this association was limited, failure to find an association between lameness and milk production may be due to type II errors. Previous studies have reported that high producing cows are more likely to become lame (Green et al., 2002; Archer et al., 2010); however, these studies were limited to lactating dairy cows. Cows with high milk yield have different time budgets and spend more time standing than lower producing cows (Norring et al., 2012). Increased standing time may increase the chances of lameness development through the development of claw lesions (Chapinal et al., 2009; Proudfoot et al., 2010). An alternative hypothesis for the lack of association between milk production and lameness during the dry period in the current study may be a result of differences in the time budget between dry cows and lactating cows. To our knowledge this hypothesis remains untested.

Although this study was not designed to measure the association between lameness onset/cure and herd-level management practices, we noted none of the farms applied measures for improving claw health to the non-lactating animals. Failure to include dry cows may have influenced the onset of lameness during the dry period. Future studies should examine the effect

of practices such as the foot baths, routine gait scoring and trimming on lameness during the dry period.

3.5 Conclusion

There was a high incidence of lameness during the dry period. Hoof trimming before the dry-period reduced the risk of lameness for primiparous, but not for multiparous cows. Low body condition at dry off and non-infectious hoof lesions in the weeks before dry-off were associated with chronic lameness during the dry period.

Chapter 4: The interplay between lameness and transition period diseases[5]

4.1 Introduction

During the transition period (i.e. ± 3 wk of calving) dairy cows are at the highest risk of developing infectious and metabolic diseases (Ingvartsen, 2006; Mulligan and Doherty, 2008; LeBlanc, 2010). Changes in metabolism (Bell, 1995; Grummer, 1995), and decreased DMI (Hayirli et al., 2002; Hayirli and Grummer, 2004) in the weeks immediately before calving have been associated with the occurrence of two common transition diseases: metritis (Hammon et al., 2006; Huzzey et al., 2007; Dubuc et al., 2010) and SCK (Goldhawk et al., 2009; Ospina et al., 2010).

Reduced DMI during the pre-calving period may prolong and aggravate the negative energy balance during the transition period (Grummer et al., 2004), resulting in fat mobilization (Weber et al., 2013) that triggers a cascade of proinflammatory processes in the adipose tissue and the liver (Sordillo et al., 2009; Contreras and Sordillo, 2011). Increased liver inflammation has been linked to higher occurrence of transition diseases (Bertoni et al., 2008). This may explain the observed association between body condition loss during the dry period and the occurrence of uterine diseases (Chebel et al., 2018) and SCK (Kaufman et al., 2016; Rathbun et al., 2017). Although the etiologies of infectious and metabolic diseases differ, it seems that DMI and body condition loss underpin some of the mechanisms that contribute to disease vulnerability.

[5] A version of this chapter has been submitted for publication: Daros, RR; Eriksson, HK; Weary, DM and von Keyserlingk, MAG. The relationship between transition period diseases and lameness, feeding time, and body condition during the dry period.

Lameness has been studied in lactating cows, but little is known about lameness during the dry period and how it relates to transition diseases. Lameness is a common (Solano et al., 2015; Randall et al., 2019) and painful condition (Whay et al., 2005; Chapinal et al., 2010) that has been associated with reduced feeding time and decreased DMI (Bach et al., 2007; Miguel-Pacheco et al., 2014; Weigele et al., 2018). Based on these findings, lameness during the dry period likely reduces feed intake, resulting in greater and prolonged negative energy balance and greater body condition loss, increasing susceptibility to disease. A study by Calderon and Cook (2011) supports this rationale, as cows diagnosed as lame during the 3 wk before calving had higher levels of BHB (a marker for SCK; Duffield, 2000) after calving. Although the association between lameness during the close-up period and transition disease is to be expected (e.g. Vergara et al., 2014), there is a dearth of information on whether lameness around dry-off is also associated with transition diseases.

The objectives of this study were to compare the incidence of metritis and SCK between cows that were lame or sound during at dry-off and to explore the relation between lameness and transition diseases through the associations between lameness with feeding time and body condition loss.

4.2 Materials and methods

This prospective longitudinal study was part of a larger project designed to study lameness epidemiology during the pre and postpartum period, and the association between lameness and transition period diseases. All participating dairy farms were located in the lower Fraser Valley region in British Columbia, Canada. Data were collected from May 2017 through

January 2018. The project was approved by the Animal Care Committee at the University of British Columbia (protocol A15-0084).

4.2.1 Sample size calculation

We hypothesized that disease incidence would be higher for cows that were lame at dry-off compared to cows that were sound. Based on previous reports on the incidence of metritis (~ 20%; Chapinal et al., 2011) we assumed a 10-point difference in incidence between lame (20%) and sound (10%) cows. For SCK, we assumed a 15-point difference (30% incidence for lame cows vs 15% incidence for sound cows), as in previous studies the incidence of SCK ranged from 20 to 40% (LeBlanc, 2010; McArt et al., 2012). Using the sample size formula for testing differences in proportions described by Dohoo et al. (2012), with power of 80% and an error rate of 5%, we estimated a sample size of approximately 400 cows (200 in each group; lame, sound) to detect a 10-point difference in metritis cumulative incidence. For SCK a sample of 242 (n=121 lame and n=121 sound) cows would be needed to detect a 15-point difference in SCK incidence. We assumed that the effect of lameness on disease incidence would be the same across farms, and we did not account for data clustering as all outcomes and predictors of interests were measured at cow level (Dohoo et al., 2012).

4.2.2 Farm and cow enrollment criteria

Farms were pre-selected through a partnership with a hoof-trimming company (AR-PE Hoof Trimming Ltd., Abbotsford, Canada); selection was based on herd size (>160 lactating cows), freestall housing, availability of individual cow records, and willingness to participate in

the study. From 9 enrolled farm, only 6 were included in this study; on the remaining 3 farms data on transition disease was not collected.

On each farm, all parous cows with expected calving date between July 21 and December 1, 2017 were enrolled 9 wk before expected calving. Cows were assessed weekly during the dry period, and twice a wk for the first 2 wk after calving. A total of 461 cows were initially enrolled, of which 34 were removed before calving. A detailed list of reasons for cow removals is presented in Figure 4.1.

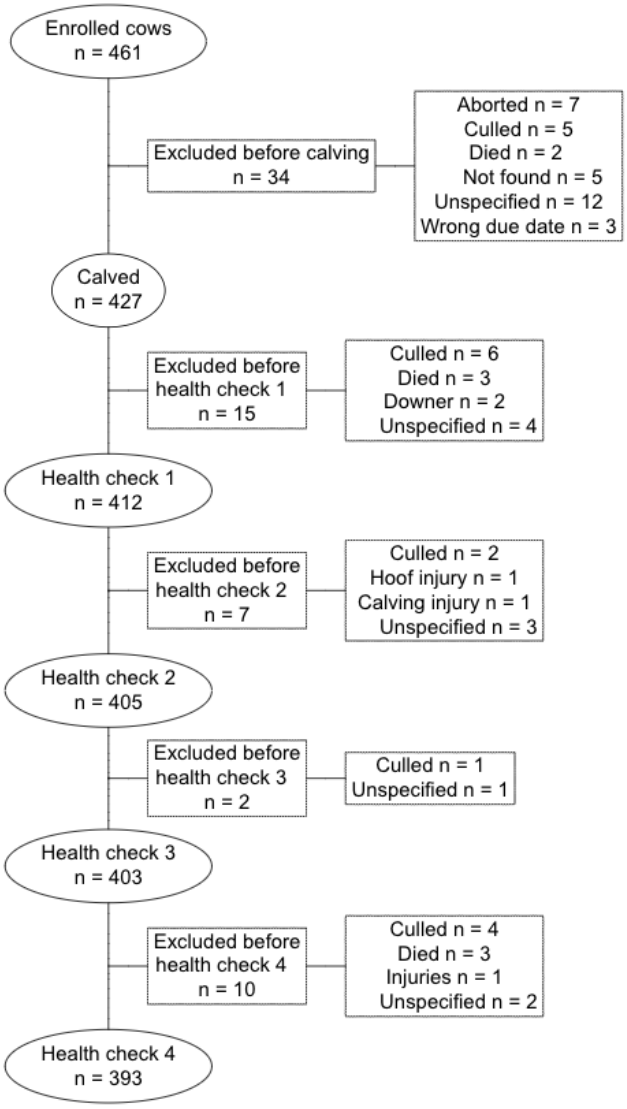

Figure 4.1 Diagram of cows removed from the study.

4.2.3 Farm data collection and description

A structured interview was conducted with the farm manager during the first visit to each farm. Key aspects about the general herd management and health management of transition dairy cows were recorded. Environmental variables such as type of flooring, number of stalls per pen, and number of feed spaces per pen were collected through environmental inspection during the first visit. Number of cows in the dry pens was recorded weekly to calculate stocking densities at the feed bunk and the lying stalls. A detailed description of the enrolled farms is presented in Table 3.1 (see previous chapter). Average herd size (mean ± SD) was 361 ± 137 lactating cows, and milk production averaged 11,866 ± 179 kg of milk per 305-d lactation.

4.2.4 Gait scoring and lameness definition

Enrolled cows were gait scored weekly using a 5-point scale described by Flower and Weary (2006). The weekly gait scores were transformed into weekly lameness status (lame or sound); lameness was defined as at least two consecutive gait scores of 3, or one gait score ≥ 4 (Eriksson et al., in review). Conversely, the cows were considered sound when two consecutive gait scores were ≤ 2.

To test our primary objective, cows were retrospectively classified as either sound or lame based on their lameness status at the first wk after enrollment (hereafter referred to as sound or lame at dry-off). When exploring which mechanisms lameness contributes to transition diseases, we established 3 different groups based on the proportion of weeks the cows were lame during the pre-calving period: *chronically lame* (cow remained lame during all pre-calving visits), *always sound* (cow remained sound during all pre-calving visits), and *other* (cow changed

lameness status during the dry period; lame to sound or sound to lame). We also created a continuous variable, expressing the proportion of weeks of lameness status as lame (see criteria for lameness status above) during the dry period.

All gait scores were performed by 2 trained observers (RRD, HKE). A detailed description of gait scoring training and interobserver reliability is reported in Eriksson et al. (in review). Live scoring interobserver agreement through weighted kappa was 0.54 and 0.55 before and after the study, respectively.

4.2.5 Body condition and body condition change (ΔBC)

Enrolled cows were body condition scored wk 8, 6, 4, 2, 1 and 0 before expected calving date, using a 5-point scale in 0.5 points increments (Ferguson et al., 1994). After calving, cows were assessed for BCS during the first wk postpartum, and again between wk 2 and 3 after calving. Due to differences between expected and actual calving date BCS was summarized as: average BCS wk 10 to 8 before calving (hereafter referred to as BCS at dry-off), and average BCS from the wk before calving to 2 d after calving (hereafter referred to as BCS at calving). We chose to average the BCS at dry-off and at calving to reduce the effect of inter-observer disagreements on the ΔBC estimates (Morin et al., 2017). BCS at dry-off and at calving were further categorized as thin (BCS < 3.0), good (BCS 3.0 to 3.5) and fat (BCS > 3.5). ΔBC during the dry period was calculated as the difference between BCS at calving and BCS at dry-off (a positive value indicates that the cow increased her BCS during the dry period). Seven cows did not have data for BCS at dry-off; for these cows BCS at dry-off was imputed using the BCS assessed on wk 5 (n = 6) and 4 (n = 1) before calving.

BCS were assigned by 4 jointly trained observers. Interobserver agreement was calculated with intra-class correlation (**ICC**), which allows for the inclusion of more than 2 non-random observers of ordinal data (Hallgren, 2012). The ICC was set for *two-way* and *agreement* methods that consider the observer to not be chosen from a random sample of observers, and that BCS scores are in perfect agreement – i.e. penalizes more if the BCS are not exactly the same across observers (Hallgren, 2012). ICC values take values from 0 (poor agreement) to 1 (excellent agreement); the calculated ICC for the 4 observers was 0.81 (95% CI: 0.73 – 0.87). The ICC was calculated across 4 observers that body condition scored each of 54 cows, from one participating farm on the same day. While scoring the cows, observers did not share information about the scores assigned. The minimum BCS assigned among the observers was 2 and the maximum was 5.

4.2.6 Feeding time

Time-lapse cameras (Cuddeback Digital® 20 megapixels Long Range IR model, De Pere, WI, USA) were installed to record the feed bunk of the dry pens on 5 farms every 10-min. In the remaining farm, 3 cameras (CCTV camera, model WVCW504SP, Panasonic, Osaka, Japan) were mounted 6 m above the feed bunk in the dry pens. These cameras were connected to a digital video surveillance system (GeoVision, GeoVision Inc., Corona, CA, USA), which recorded video continuously. Videos were scan sampled every 10-min to evaluate feeding time. A 10-min scan sampling protocol has been previously validated for feeding time of feedlot cattle (Mitlöhner et al., 2001).

Enrolled cows were individually marked with alphanumeric symbols on their backs, to facilitate recognition of the animals. Coat color on the back (% of black), and face markings

were also recorded to facilitate recognition. Differences in cow size on one farm made it impossible to determine if small cows were present at the feed bunk. For that reason, a random sample of focal cows (n = 159) were selected from the other 5 farms after the on-farm data collection was completed.

A focal cow was scored as present at the feed bunk if the head was fully over the feed bunk (i.e. ears past the feeding barrier). Daily feeding time in minutes was derived by multiplying the number of images that the focal animal was present during a 24-h period by 10. Daily feeding time was recorded for each focal cow once per wk from wk 8 to 1 before calving. Because of technical issues (e.g. drained camera batteries), or because the cow was moved to another pen (e.g. to the maternity or hospital pen), not all cows had weekly measures of feeding time. On average, the focal cows had feeding time data for 5.6 ± 1.5 d. A total of 8337 images (from 23 focal cows from 4 farms) were evaluated for the presence of focal cows by 5 trained observers. The same statistical method as described above for BCS was applied. Calculated ICC for the 5 observers was 0.96 (95% CI: 0.94 – 0.97).

4.2.7 Transition period diseases

Enrolled cows were examined every 3 to 4 d for puerperal metritis (hereafter referred to as metritis) and SCK from d 3 to d 17 after calving (in total 4 health checks per animal). Metritis was scored 0 to 4 according to the consistency, smell and presence of pus in cows' vaginal discharge (VD; clear VD = 0, VD < 50% pus, no fetid smell = 1, VD > 50% pus, no fetid smell = 2, purulent VD with foul smell = 3, red/brown watery fetid smell VD = 4). As defined by Sheldon et al. (2006) only cows with metritis score = 4 were considered metritic. SCK was diagnosed with a cow side blood test (BHB ≥ 1.2mmol/L; Duffield et al., 2009), using hand-held

FreeStyle Precision Neo ketone monitoring system meters (Abbot diabetes care Ltd., Witney, UK; validated by Macmillan et al. 2017). We have not assessed cows for clinical ketosis, however it is likely that some cows with high levels of BHB were experiencing clinical ketosis and only classified as SCK. Information about RP, and treatment of hypocalcemia and DA during the first 17 DIM was retrieved from farm records.

Data collected during the health checks were summarized per cow; cows were considered positive for metritis and SCK if they scored positive for these diseases on at least 1 health check, regardless if they had another disease or not. Cows that had less than 3 health checks and were negative for metritis or SCK were assigned missing values for these diseases. Disease data were further binary categorized as either being healthy, or developing a TD – transition disease, which included any of or the combination of the following conditions: SCK, metritis, treatment of RP, treatment of hypocalcemia or treatment of DA during the first 17 DIM.

4.2.8 Other cow variables

The animals were categorized as primiparous or multiparous based on their parity at dry-off. Because we reported individual data from the prepartum and postpartum, when referring to data from the postpartum period parity is referred to as 2nd lactation (for the primiparous at dry-off), and 3+ lactation animals (for the multiparous cows at dry-off). Individual previous last lactation 305-d corrected milk yield in kg and dry-off date were retrieved from farm records. Previous milk yield was centered and scaled in reference to the mean of previous lactational milk yield of all enrolled cows, thus the values used in analyses represent the SD from the mean.

4.2.9 Statistical analyses

All statistical analyses were performed in R 3.5.2 (R Core Team, 2019) using the Rstudio interface (RStudio Team, 2016). List of R statistical packages used, full statistical analyses code and output, and data used for the analysis are available online at https://doi.org/10.5683/SP2/Q4NL1C.

All multilevel logistic regression models were fitted through maximum likelihood using adaptive Gauss-Hermite quadrature method with 12 points, to better estimate model parameters (Pinheiro and Chao, 2006). The assumption of linearity between continuous predictors and the log-odds of the outcome variables were assessed graphically. All multilevel linear regression models were fitted through restricted maximum likelihood. For these linear models, normality and homoscedasticity of lower levels residuals were assessed through residual and quantile-quantile plots.

In all models, confidence intervals of model parameters were estimated with the profile likelihood method (Venzon and Moolgavkar, 1988). Multicollinearity was tested with a variation inflation factor; no variables had a variation inflation factor greater than 10 (Dohoo et al., 2012). Plausible biological interactions were tested and kept in the model if $P < 0.10$. When inclusion of interaction terms resulted in model convergence failure we explored the interaction in a separate model (detailed below).

4.2.9.1 Hypothesis testing

We tested the association between lameness at dry-off (sound vs. lame) and the occurrence of metritis, SCK or TD, controlling for known confounders (e.g. parity). For this we built 3 multilevel logistic regression models with farm as random intercept – one for each outcome variable: metritis, SCK and TD. In these models, we included parity, previous lactation

milk production, ΔBC, BCS at calving and lameness at dry-off as predictors. In the model for metritis, RP was also included as predictor. Based on our causal diagram (Figure 2), ΔBC should be considered an intervening variable for the association between lameness and transition disease, and therefore not be included in the models. However, in a preliminary analysis we found that lameness (lameness at dry-off, lameness group or proportion of weeks lame) was not associated with ΔBC – allowing us to include ΔBC in the models. More details on which variables were associated with ΔBC are presented below.

4.2.9.2 Exploratory analyses

We also built models for metritis, SCK and TD similar to those described above, but instead of using lameness at dry-off as predictors, we used lameness group as a predictor in one set of models and proportion of weeks lame during the dry period as predictor in another set of models. These models allowed us to explore the associations between chronic lameness and transition disease, and the cumulative effect of lameness during the dry period on the incidence of transition diseases. In the models for SCK, the interaction between lameness group and BCS at calving did not converge. To remove the interaction term, we built separate models to measure the association between lameness and SCK using a subset of data containing only data from fat cows (calving BCS > 3.5). The rationale for this analysis was based on the premise that lameness would contribute more to SCK risk between fat cows than to cows that have lower body

condition, as low body condition seems to be a protective factor for SCK (Duffield et al., 1998).

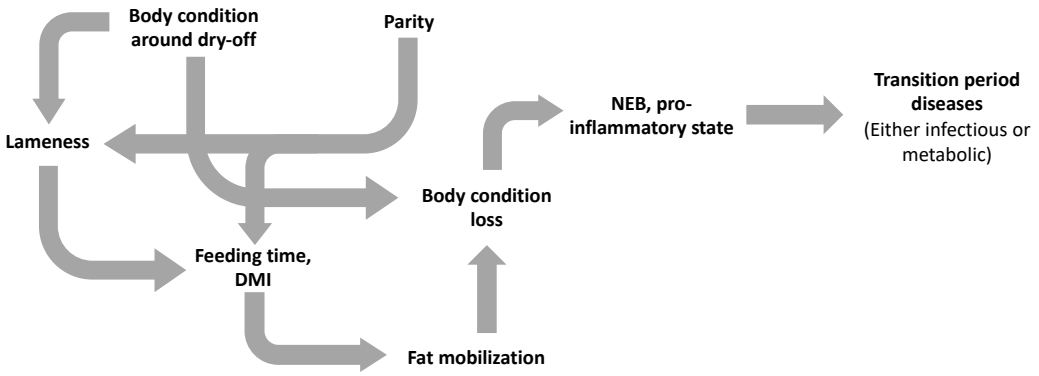

Figure 4.2 Causal diagram showing the hypothesized causal web linking lameness to transition period disease.

In our causal diagram (Figure 4.2) we proposed that lameness reduces feeding time, and that reduced feeding time (especially during the 3 weeks before calving) increases the risk of transition diseases. Hence, we build models to test these different parts of the causal diagram. To measure the association between weekly lameness status and weekly feeding time we built a multilevel linear regression model, including the random intercepts of farm and cow within farm, and using wk in relation to calving as random slope. Feeding time (min/d) was used as the outcome variable, while lameness status (lame vs. sound), parity, BCS at dry-off, previous lactation milk production, and period (far-off = wk 8 to 4 before calving, and close-up = wk 3 before calving to the wk of calving) were included as predictors. Period was included as a predictor because feeding time during the dry period changes at a different rates in the far-off and close-up periods (Grummer et al., 2004).

To assess the relationship between feeding time and the occurrence of disease (metritis, SCK and TD) we built multilevel logistic regression models similar to what is described above; in these models, lameness and ΔBC were considered intervening variables and therefore not included. Predictors for these models included: feeding time, average per cow using the data

available for the 3 wk before calving, parity, primiparous vs. multiparous, BCS at calving, thin, good and fat, and previous milk production. BCS at calving did not converge due to the low number of thin cows in the dataset; BCS at calving was recoded as fat (BCS >3.5) and not fat (BCS ≤ 3.5).

We also built a model to assess the factors associated with ΔBC. Because ΔBC was a continuous variable with no repeated measure (i.e. only one measure per cow) we fitted two linear multilevel model using farm as random effect to: 1) measure the associations between ΔBC and lameness group (always sound, chronically lame and other), parity, BCS at dry-off, previous milk production and number of days dry, and 2) measure the association between ΔBC and feeding time, that also included parity, BCS at dry-off, previous milk production and days dry. For this model, feeding time was averaged per cow across the dry period. There was a non-linear association between feeding time and ΔBC (Figure 4.3); thus, to improve model fit we created a variable splitting feeding time in two categories, low (average feeding time ≤ 4h/d) and high (average feeding time > 4h/d). To improve model fit and test the difference in the slopes of feeding time between low and high feeding time, the interaction between continuous feeding time and categorical feeding time was included in the model.

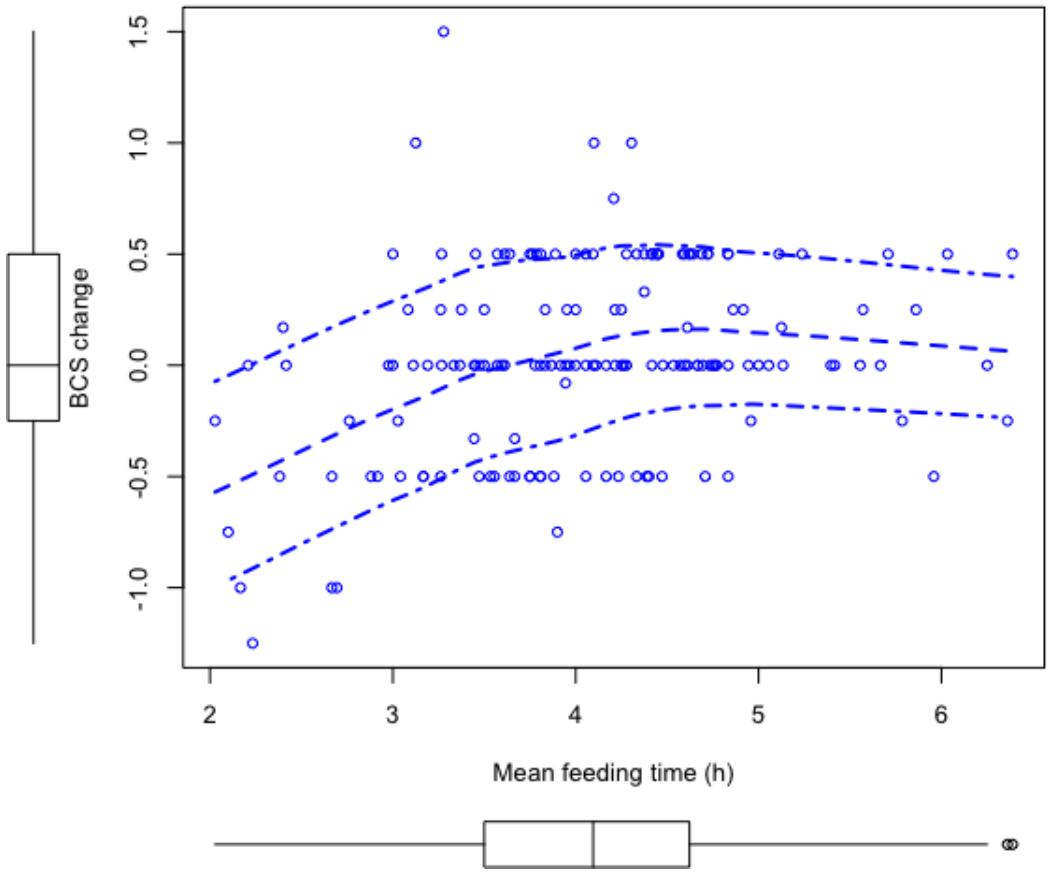

Figure 4.3 Relationship between BCS change and average daily feeding time

We extracted the residuals from a multilevel logistic regression model that included farm as random effect, the fixed effects of average feeding time in the close-up period, parity, BCS at calving, ΔBC and previous lactation milk yield and the response variable TD. This approach allowed us to explore the residual variation – i.e. unexplained variation from our model, which comes from unmeasured effects plus errors in measured effects. Hence, to test if lameness group (always sound, chronically lame or other) explain some of this residual variation in TD, we fitted a univariable linear regression, using the model residuals as outcome and lameness group as predictor. This simple linear regression allowed us to measure model's correlation coefficient

(R^2), quantifying how much extra variation lameness is explaining after controlling for mediating and confounding variables. Although this strategy has been criticized for yielding unreliable estimates for comparing groups (García-Berthou, 2001), this approach is justifiable when the objective is to gain deeper understanding of the data instead of comparing means. Furthermore, we also tested a full model including farm as random effect, the fixed effects of lameness group, average feeding time in the close-up period, parity, BCS at calving, ΔBC and previous lactation milk yield and the response variable TD. This model indicated that chronic lameness is associated with increased odds of TD (OR: 3.8; 95% CI: 1.1 – 12.9; P = 0.03). Further details on this model can be found online [https://doi.org/10.5683/SP2/Q4NL1C] as supplementary material and will not be reported further.

4.3 Results

The proportion of cows that were chronically lame, always sound or that changed lameness status during the dry period was 23, 33 and 43%, respectively. Overall disease incidence and disease incidence per lameness group are presented in Table 4.1. Cows spent in average 245 ± 53 min/d feeding. Feeding time in relation to lameness status is presented on Figure 4.4.

Table 4.1 Incidence of transition period diseases in the first 3 weeks after calving by lameness group and by parity in 427 dairy cows in 6 dairy farms in the lower Fraser Valley region in British Columbia, Canada.

Disease	Overall incidence	Incidence per lameness group		
		Always sound	Chronically lame	Other [5]
SCK [1]	35 %	31 %	41 %	35 %
Metritis [2]	28 %	21 %	39 %	28 %
RP [3]	10 %	10 %	11 %	9 %
Hypocalcemia [3]	5 %	4 %	8 %	4 %
DA [3]	3 %	0 %	4 %	3 %
Any transition disease [4]	54 %	46 %	66 %	54 %

[1] Positive if blood BHB ≥ 1.2mmol on at least 1 health check.
[2] Positive if VD was red/brown, watery and fetid smell on at least 1 health check.
[3] As per farm records.
[4] Any of the following: SCK, metritis, RP, hypocalcemia or DA within the first 3 weeks after calving.
[5] Cows that changed their lameness status during the dry period.

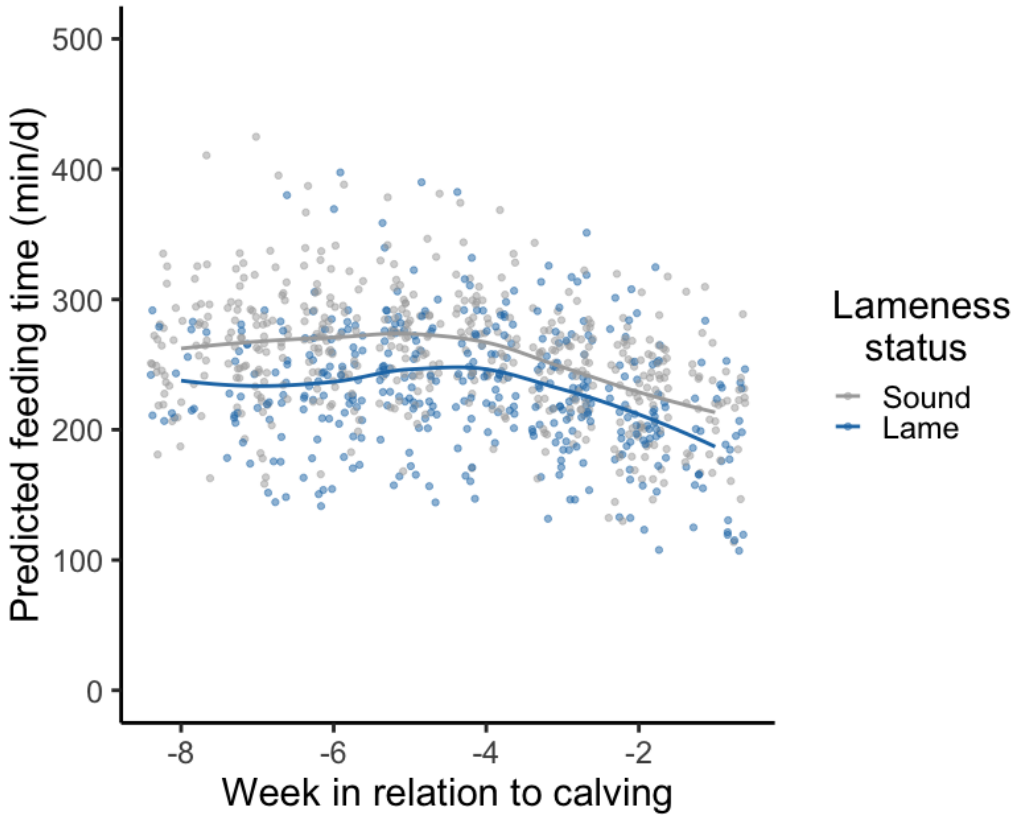

Figure 4.4 Time spent feeding in relation to week before calving by lameness status.
Each dot represents the estimated feeding time of each cow each week. Dots were jittered through geom_jitter function and lines are estimated through loess method from the geom_smooth function (ggplot2 package; Wickham, 2016)

4.3.1 Hypothesis testing

Parameters estimates and confidence intervals for factors associated with metritis, SCK and TD are presented in Table 4.2. Cows that were lame (vs. sound) at dry-off had higher odds of metritis and TD postpartum, but not SCK. Cows in their 3rd or later lactation had higher odds of SCK and TD, but not metritis, compared with cows in their 2nd lactation. An increase in BCS from dry-off to calving was associated with reduced odds of metritis, SCK and TD. BCS at calving was only associated with the odds of SCK. Thin cows (BCS < 3.0) and cows with good BCS (3.0 to 3.5) at calving had lower odds of SCK compared to fat cows (BCS > 3.5). Previous lactation milk production was not associated with the odds of metritis, SCK or TD. Cows with RP after calving had higher odds of metritis compared with cows that were not diagnosed with RP.

Table 4.2 Parameters from the models for the association between lameness at dry-off and the occurrence of metritis, SCK and any transition disease within the first 3 weeks after calving in 403 dairy cows in 6 dairy farms in the lower Fraser Valley region in British Columbia, Canada.

Predictor	Metritis[1]				SCK[2]				Any transition disease[3]			
	Odds ratio	Lower 95%CI	Upper 95%CI	*P*	Odds ratio	Lower 95%CI	Upper 95%CI	*P*	Odds ratio	Lower 95%CI	Upper 95%CI	*P*
Lameness at dry-off												
Sound	Ref.	-	-	-	Ref.	-	-	-	Ref.	-	-	-
Lame	1.9	1.12	3.11	0.02	1.13	0.70	1.83	0.62	1.82	1.15	2.92	0.01
Parity												
2nd lactation	Ref.	-	-	-	Ref.	-	-	-	Ref.	-	-	-
3 or more lactation	0.86	0.49	1.51	0.60	2.58	1.53	4.42	<0.01	1.81	1.11	2.97	0.02
ΔBC[4]	0.46	0.23	0.90	0.02	0.36	0.19	0.68	<0.01	0.47	0.26	0.86	0.01
BCS at calving												
BCS > 3.5	Ref.	-	-	-	Ref.	-	-	-	Ref.	-	-	-
BCS 3.0 to 3.5	1.15	0.65	2.07	0.64	0.67	0.40	1.12	0.13	0.67	0.40	1.12	0.13
BCS < 3.0	1.10	0.24	4.75	0.90	0.07	0.00	0.44	<0.01	0.39	0.11	1.36	0.13
Previous lactation milk yield[5]	1.17	0.88	1.58	0.28	0.83	0.63	1.09	0.18	0.91	0.70	1.19	0.50
RP[6]												
No	Ref.	-	-	-								
Yes	9.70	4.42	22.58	<0.01								
Random intercept												
Farm	Variance: 0.38				Variance: 0.35				Variance: 0.29			

[1] Positive if VD was red/brown, watery and with fetid smell on at least 1 health check.
[2] Positive if blood BHB ≥ 1.2mmol on at least 1 health check.
[3] Any of the following: SCK, metritis, RP, hypocalcemia, DA.
[4] ΔBC = Change in BCS from dry-off to calving, 1-unit change equals cow gained 1 BCS point over the dry period.
[5] Scaled variable: 1 unit change equals to change in 1 SD from mean previous lactation milk production from all enrolled cows.
[6] RP was not included as predictor in the models for SCK and any transition disease. RP data was collected through farm records.

4.3.2 Exploratory data analyses

4.3.2.1 Lameness and metritis

Results from the models evaluating lameness as a predictor for metritis occurrence are presented in Table 4.3. There was a tendency for chronically lame cows to have higher odds of developing metritis compared to cows that remained sound. This finding is supported by the linear relation between proportion of weeks lame and odds of metritis; for each 10% increase in weeks lame the odds of metritis increased by 1.07 times. Cows that gained BCS from dry-off to calving had reduced odds of metritis. BCS at calving, parity and previous lactation milk production were not associated with the odds of metritis. RP was associated with increased odds of metritis. No interactions were retained in any of the models for metritis.

Table 4.3 Parameters from the models evaluating lameness as a predictor for metritis in 403 dairy cows from 6 dairy farms in the lower Fraser Valley region in British Columbia, Canada.

Predictor	Lameness group				Proportion of weeks lame			
	Odds ratio	Lower 95%CI	Upper 95%CI	*P*	Odds ratio	Lower 95%CI	Upper 95%CI	*P*
Lameness group								
Always sound	Ref.	-	-	-				
Chronically lame	1.85	0.91	3.75	0.09				
Other[1]	1.50	0.83	2.73	0.18				
Proportion of weeks lame					1.00[4]	1.00	1.01	0.05
Parity								
2nd lactation	Ref.	-	-	-	Ref.	-	-	-
3 or more lactation	0.84	0.48	1.49	0.56	0.83	0.47	1.48	0.53
ΔBC[2]	0.47	0.24	0.92	0.03	0.47	0.24	0.94	0.03
BCS at calving								
BCS > 3.5	Ref.	-	-	-	Ref.	-	-	-
BCS 3.0 to 3.5	1.15	0.65	2.08	0.64	1.14	0.64	2.06	0.66
BCS < 3.0	1.02	0.22	4.46	0.98	1.05	0.23	4.60	0.95
Previous lactation milk yield[3]	1.17	0.87	1.57	0.29	1.17	0.87	1.57	0.29
RP								
No	Ref.	-	-	-	Ref.	-	-	-
Yes	10.43	4.74	24.46	<0.01	10.16	4.63	23.67	<0.01
Random intercept								
Farm	Variance: 0.41				Variance: 0.41			

[1] Cows that changed their lameness status during the dry period
[2] ΔBC = Change in BCS from dry-off to calving, a 1-unit increase means that the cow gained 1 BCS point over the dry period.
[3] Scaled variable: 1-unit change equals to change in 1 SD from mean previous lactation milk production from all enrolled cows.
[4] Adjusted odds for each 10% increase in weeks lame during the dry period = 1.07.

4.3.2.2 Lameness and SCK

Results from the models evaluating lameness during the dry-period as a predictor for SCK are presented in Table 4.4. Neither proportion of weeks lame during the dry-period, nor chronic lameness during the dry-period were associated with higher odds of SCK. Gaining BCS during the dry period was associated with reduced odds of SCK, regardless of BCS at calving. Cows that were thin at calving had reduced odds of SCK compared to cows that were fat. Animals in their 3rd or later lactation had higher odds of having SCK compared to cows in their 2nd lactation.

When only considering fat cows at calving (n = 108), chronically lame cows tended to have higher odds of SCK (OR: 3.44; CI: 1.00 to 13.36; *P* = 0.06) compared to cows that remained sound. In this model neither ΔBC (OR: 0.57; CI: 0.18 to 1.83; *P* = 0.34), parity (OR: 2.17; CI:

0.75 to 6.63; $P = 0.16$) nor previous milk production (OR: 0.69; CI: 0.43 to 1.08; $P = 0.11$) were associated with the odds of SCK.

Table 4.4 Parameters from the models evaluating lameness as a predictor for SCK[1] in 404 dairy cows in 6 dairy farms in the lower Fraser Valley region in British Columbia, Canada.

Predictor	Lameness group				Proportion of weeks lame			
	Odds ratio	Lower 95%CI	Upper 95%CI	*P*	Odds ratio	Lower 95%CI	Upper 95%CI	*P*
Lameness group								
Always sound	Ref.	-	-					
Chronically lame	1.62	0.84	3.15	0.15				
Other[2]	1.28	0.75	2.20	0.38				
Proportion of weeks lame	-	-	-	-	1.00[4]	1.00	1.01	0.13
Parity								
2nd lactation	Ref.	-	-	-	Ref.	-	-	-
3 or more lactation	2.39	1.40	4.13	<0.01	2.37	1.39	4.10	<0.01
ΔBC[1]	0.37	0.19	0.68	<0.01	0.37	0.19	0.68	<0.01
BCS at calving								
BCS > 3.5	Ref.	-	-	-	Ref.	-	-	-
BCS 3.0 to 3.5	0.65	0.39	1.09	0.10	0.64	0.38	1.08	0.10
BCS < 3.0	0.06	0.00	0.41	0.01	0.06	0.00	0.42	<0.01
Previous lactation milk yield[3]	0.84	0.63	1.10	0.20	0.84	0.63	0.10	0.20
Random intercept								
Farm	Variance: 0.38				Variance: 0.39			

[1] Positive if blood β-hydroxy-butyrate ≥ 1.2mmol on at least 1 health check; ΔBC = Change in BCS from dry-off to calving, 1-unit change equals cow gained 1 BCS point over the dry period.
[2] Cows that changed their lameness status during the dry period.
[3] 1-unit change equals to change in 1 SD from mean previous lactation milk production from all enrolled cows.
[4] Adjusted odds for each 10% increase in weeks lame during the dry period = 1.05.

4.3.2.3 Lameness and transition disease

Results from the models evaluating lameness during the dry-period as a predictor for TD are presented in Table 4.5. Chronically lame cows had higher odds TD compared to animals that remained sound. For each 10% increase in the proportion of weeks lame, the odds of TD increased by 1.09 times. Cows with 3 or more lactation had increased odds of TD compared to 2nd lactation animals, while cows that gained BCS during the dry-period had reduced odds of TD. BCS at calving and previous lactation milk production were not associated with the odds of TD. No interactions were retained in any of the models for TD.

Table 4.5 Parameters from the models evaluating lameness as a predictor for any transition disease[1] in 404 dairy cows in 6 dairy farms in the lower Fraser Valley region in British Columbia, Canada.

Predictor	Lameness group				Proportion of weeks lame			
	Odds ratio	Lower 95%CI	Upper 95%CI	*P*	Odds ratio	Lower 95%CI	Upper 95%CI	*P*
Lameness group								
Always sound	Ref.	-	-					
Chronically lame	2.22	1.19	4.22	0.01				
Other[2]	1.48	0.90	2.44	0.12				
Proportion of weeks lame	-	-	-	-	1.01[4]	1.00	1.01	<0.01
Parity								
2nd lactation	Ref.	-	-	-	Ref.	-	-	-
3 or more lactation	1.73	1.06	2.86	0.03	1.70	1.04	2.81	0.04
ΔBC[3]	0.49	0.27	0.88	0.02	0.49	0.27	0.88	0.02
BCS at calving								
BCS > 3.5	Ref.	-	-	-	Ref.	-	-	-
BCS 3.0 to 3.5	0.67	0.39	1.12	0.12	0.66	0.39	1.10	0.11
BCS < 3.0	0.35	0.10	1.23	0.10	0.37	0.10	1.37	0.12
Previous lactation milk yield[4]	0.91	0.69	1.18	0.48	0.91	0.69	1.18	0.48
Random intercept								
Farm	Variance: 0.38				Variance: 0.32			

[1] Any of the following: SCK, metritis, RP, hypocalcemia, DA within 3 of calving.
[2] Cows that changed their lameness status during the dry period.
[3] ΔBC = Change in BCS from dry-off to calving, 1-unit increase equals cow gained 1 BCS point over the dry period.
[4] Scaled variable: 1-unit change equals to change in 1 SD from mean previous lactation milk production from all enrolled cows.
[5] Adjusted odds for each 10% increase in weeks lame during the dry period = 1.09.

4.3.2.4 Lameness and feeding time

Lameness was associated with reduced feeding time; during the weeks when categorized as lame cows spent on average 20 min/d (CI: -30 to -10 min/d; $P < 0.01$) less time feeding than when sound (Figure 4.4). Also, multiparous cows spent less time feeding (-19 min/d; CI: -36 to -3 min/d; $P = 0.02$) compared to primiparous cows. Cows in good BCS spent 33 min/d (CI: 16 to 49 min/d; $P < 0.01$) more time feeding, while thin cows spent 35 min/d (CI: 6 to 63 min/d; $P < 0.01$) more time feeding than fat cows. For each 1-unit increase in SD of previous lactation milk production, cows spent an extra 6 min/d (-1 to 13min/d; $P = 0.09$) feeding. There was an interaction (see Figure 4.4) between wk in relation to calving and period (far-off and close-up). During the close-up period, feeding time decreased at a greater rate (-19 min/wk; CI: -27 to -10

min/wk; $P < 0.01$) than during the far-off period (-1.7 min/wk; CI: -7.9 to 3.6 min/wk; $P = 0.55$). There was no interaction between lameness status, wk to calving and period.

4.3.2.5 Feeding time and transition diseases

Average feeding time during the close-up period (OR: 0.8; CI: 0.5 to 1.1; $P = 0.20$), parity (3rd or more lactation; OR: 0.8; CI: 0.3 to 2.0; $P = 0.64$), BCS (≤ 3.5; OR: 1.0; CI: 0.4 to 2.8; $P = 0.98$) and previous lactation milk production (OR: 0.9; CI: 0.6 to 1.4; $P = 0.73$) were not associated with metritis.

For each 1-h increase in average feeding time during the close-up period, the odds of SCK decreased by 0.7 times (CI: 0.4 to 0.9; $P = 0.02$), while cows in their 3+ lactation had higher odds of SCK (OR: 4.2; CI: 1.7 to 12.2; $P < 0.01$) compared with cows in their 2nd lactation. BCS at calving (≤ 3.5; OR: 0.9; CI: 0.4 to 2.1; $P = 0.76$) and previous milk production (OR: 0.8; CI: 0.5 to 1.1; $P = 0.23$) were not associated with the odds of SCK.

A 1-h increase in average feeding time during the close-up period also reduced the odds of TD by 0.7 times (CI: 0.5 to 1.0; $P = 0.05$). Parity 3 or higher (3rd OR: 1.5; CI: 0.7 to 3.4; $P = 0.30$), BCS (≤ 3.5; OR: 0.6; CI: 0.3 to 1.4; $P = 0.24$) and previous lactation milk production (OR: 0.9; CI: 0.6 to 1.2; $P = 0.45$) were not associated with changes in odds of TD. The trends of feeding time during the dry period by transition disease are presented on Figure 4.5.

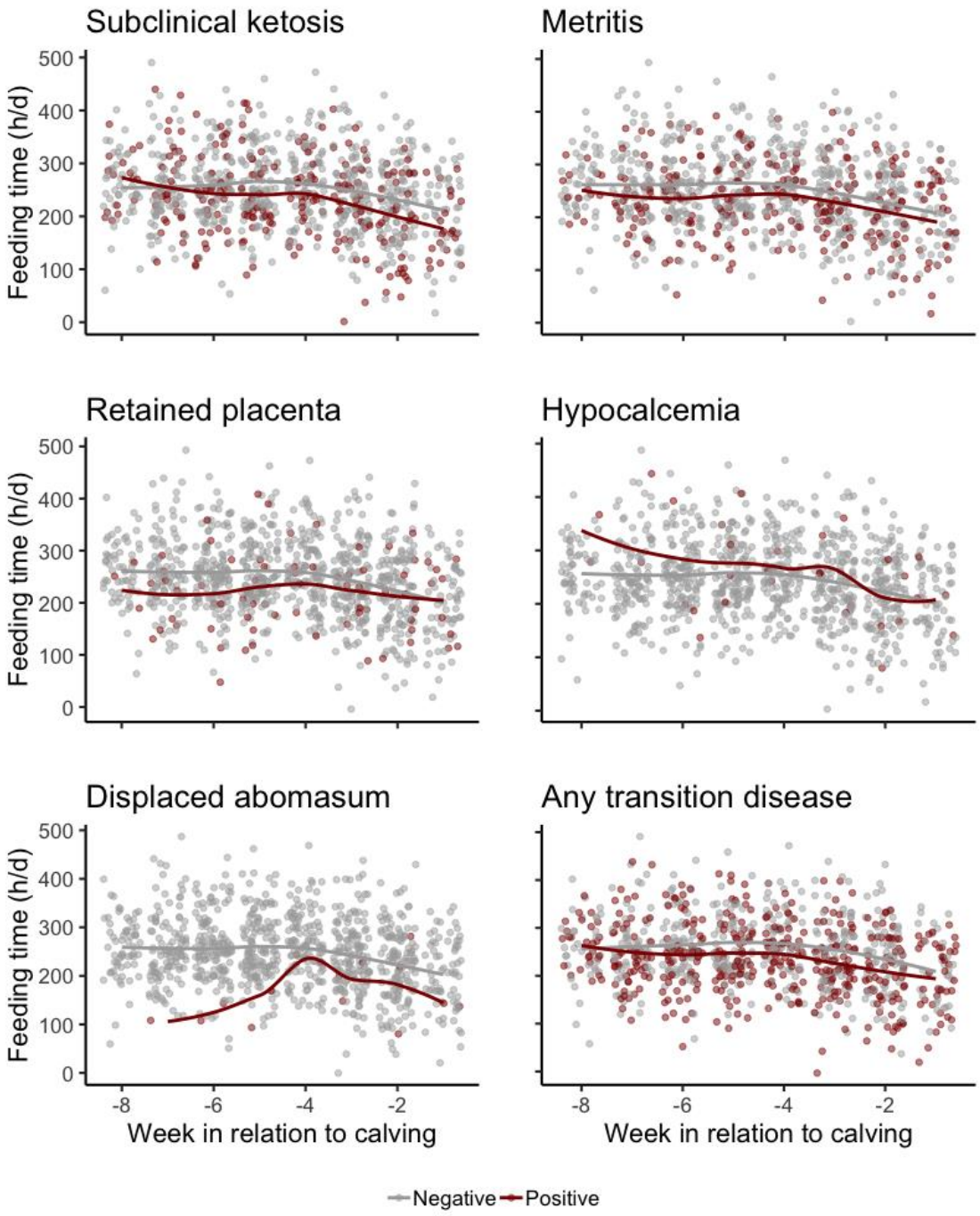

Figure 4.5 Feeding time during the dry period by transition disease category from 159 dry cows. Cows from 5 commercial freestall dairy farms located in the lower Fraser Valley in British Columbia, Canada. Each dot represents the original feeding time of each cow each week. Dots were jittered through geom_jitter function and lines are estimated through loess method from the geom_smooth function (ggplot2 package; Wickham, 2016).

4.3.2.6 Lameness and transition diseases after controlling for covariates

When modelling the residuals from the multilevel logistic regression on TD, lameness group (always sound, chronically lame and other) further explained residual variance (model statistics: $F = 3.14$ on 2 and 151 DF; $P = 0.05$; adjusted $R^2 = 0.03$).

4.3.2.7 Factors associated with changes in body condition

There was no clear association between lameness group and ΔBC (see Figure 4.6). Compared to cows that remained sound during the dry period, the ΔBC for chronically lame cows and cows that changed lameness status was -0.7 BCS points (CI: -0.17 to 0.02 BCS points; $P = 0.12$) and -0.7 BCS points (CI: -0.15 to 0.01; $P = 0.08$), respectively. Cows that were thin, and cows that had good BCS at dry-off, gained BCS (0.7; CI: 0.5 to 0.8 BCS points; $P < 0.01$ and 0.4; CI: 0.3 to 0.4 BCS points; $P < 0.01$, respectively) during the dry period compared to cows that were fat at dry-off; see Figure 4.6. For each day dry, cows increased their BCS 0.003 points (CI: 0.001 to 0.006 BCS points; $P < 0.01$). Parity (multiparous: 0.1; CI: -0.6 to 0.09 BCS points; $P = 0.76$) and previous lactation milk production (-0.007; CI: -0.05 to 0.03; $P = 0.75$) were not associated with ΔBC.

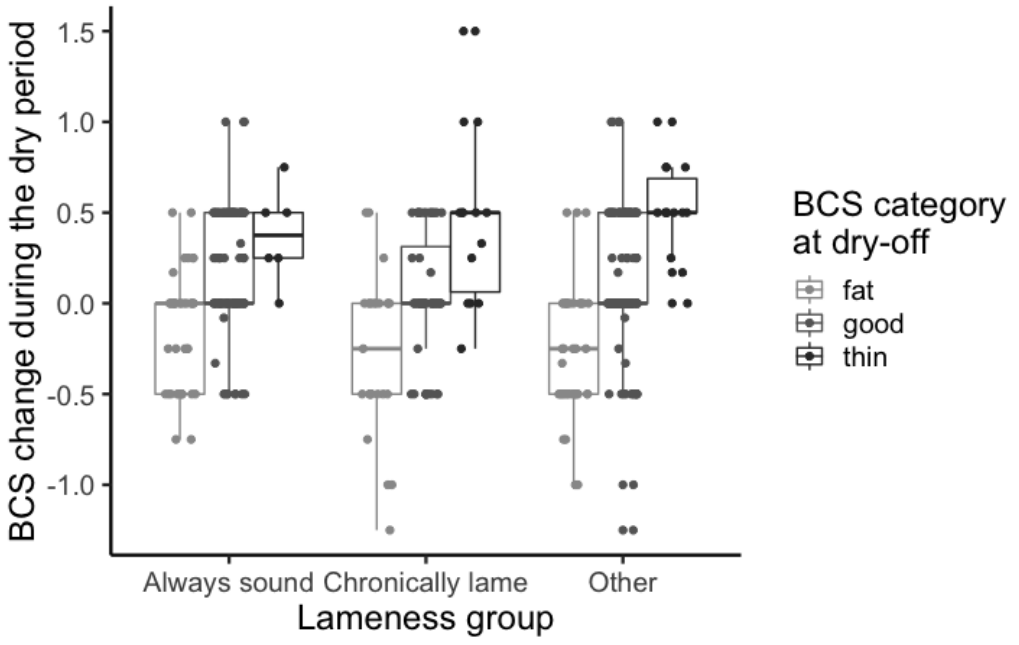

Figure 4.6 Boxplot showing ΔBC during the dry period depending on BCS at dry-off and lameness group. A total of 426 dry cows on 6 commercial freestall dairy farms in the lower Fraser Valley in British Columbia, Canada were assessed. Each dotΔBC Each dot represents the original value for each cow. BCS was categorized as fat (> 3.5), good (3.0 to 3.5) and thin (< 3.0). Lameness = other, include all cows that changed lameness status during the dry-period.

Feeding time was positively associated with ΔBC when average feeding time was ≤ 4 h/d (an extra hour feeding was associated with an 0.2 point increase in ΔBC (CI: 0.01 to 0.4; P = 0.05); see Figure 4.7) while for average feeding time > 4 h/d no relationship was found between feeding time and ΔBC (-0.08 points; CI -0.7 to 0.7; P = 0.22). Cows that were thin and cows with good BCS at dry-off gained BCS (0.8 points; CI: 0.6 to 1.1; P < 0.01 and 0.4 points; CI: 0.2 to 0.4; P < 0.01, respectively) through the dry period compared to fat cows. Number of days dry (0.003; CI: -0.00 to 0.01; P = 0.11), parity (multiparous: -0.06; CI: -0.2 to 0.1; P = 0.35) and previous lactation milk production (0.02; CI: -0.05 to 0.08; P = 0.52) were not associated with ΔBC.

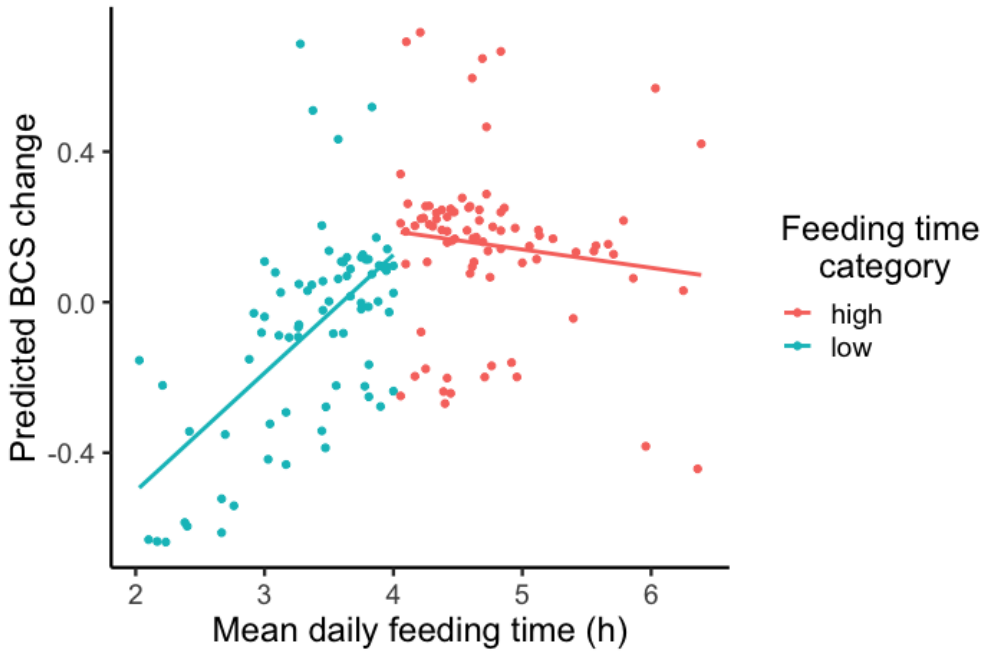

Figure 4.7 Relationship between predicted BCS change and average feeding time (h) by feeding category

4.4 Discussion

Our results support the hypothesis that lameness during the dry period is associated with transition diseases. Lameness identified 2 mo before calving was associated with an increased risk of transition disease, highlighting the importance of screening cows for lameness around dry-off.

We partially explored the causal mechanism through which lameness relates to transition health as proposed by Calderon and Cook (2011); namely, that lameness reduces feeding time and consequently DMI. In a similar study to ours, researchers from Germany also found that cows decreased their feeding time, albeit three times higher (-65 min/d when lame) than in our study (-19 min/d when lame) when they became lame (Grimm et al., 2019). The differences in feeding time between their study and ours may be due to reporting differences (their study only

reported raw values rather than predicted values), breed difference (Simmental cows vs Holstein cows) or due to differences in lactational stages (lactating vs non-lactating cows). A cross sectional study by González et al., (2008) reported a reduction of 19 min/d in average feeding time of lame cows compared to non-lame cows. However, they only included severe lameness cases; whereas, we included milder cases of lameness. Another study comparing non-lame with moderately lame cows also found that lame animals had reduced feeding time but no effect size was reported (Weigele et al., 2018). Together these studies highlight that lameness is associated with reduced feeding time, suggesting that lame cows also have lower DMI (Bach et al., 2007). Although, reduced feeding time does not always results in lower DMI (e.g. Grimm et al., 2019) some studies have shown that increased feeding time and increased DMI are correlated (e.g. Johnston and DeVries, 2018).

Reduced feeding time during the weeks before calving was associated with SCK and TD but not metritis. The lack of association between feeding time and metritis contradicts previous findings (Urton et al., 2005; Huzzey et al., 2007). Recently, Neave et al. (2018), conducted a study in the same experimental farm as the one used for the study of Urton et al., (2005) and Huzzey et al. (2007), also failed to corroborate their findings. Neave et al. (2018), argue that the differences in findings could be due to the fact that on the work of Huzzey et al. (2007) cows were not screened for SCK, which could have confounded the results since SCK has been associated with changes in feeding activity (Goldhawk et al., 2009). To explore the hypothesis proposed by Neave et al. (2018) we included (data not shown) SCK as a predictor in the metritis and feeding time model and we were not able to detect any association between feeding time and metritis incidence even when controlling for SCK. Huzzey et al. (2007) did not report if cows were diagnosed for lameness, while Neave et al. (2018) only included sound cows in their

analysis. We have not included lameness as a predictor in our model for metritis and feeding time because feeding time would then become an intervening variable on the association between lameness and metritis. However, our results showing that lame cows have lower feeding time, may partially explain the discrepancy between the results reported by Neave et al. (2018) and Huzzey et al. (2007); Huzzey et al. (2007) did not screen cows for lameness on their study. Regarding SCK, our results are in line with those reported by Goldhawk et al., (2009), who reported that cows feeding less before calving were more likely to develop SCK. Neave et al. (2018) did not find differences in feeding time during the pre-calving period for cows with both SCK and metritis compared to healthy cows. Although we have not specifically combined SCK and metritis, TD cows spent less time feeding during the dry period.

Uterine diseases and SCK have been associated with BCS loss during the dry period (Kaufman et al., 2016a; Chebel et al., 2018), which in turn is associated with BCS around dry off (Chebel et al., 2018). In our study, in addition to corroborating previous findings that changes in BCS are associated with BCS at dry-off (Hoedemaker et al., 2009; Chebel et al., 2018), we were able to evaluate the association between lameness and body condition loss during the dry period; to our surprise lameness was not associated with body condition loss. Some have suggested that cows naturally change BCS through homeorhetic mechanisms (Bauman and Currie, 1980; Roche et al., 2009), which, we speculate, may have been more influential on body condition changes than that of lameness. Based on our findings, and the ones described by Chebel et al, (2018), it seems that monitoring BCS throughout lactation with the goal of achieving moderate BCS at dry-off may be beneficial for transition health. This strategy has been tested in grazing cows by comparing cows that had their BCS experimentally manipulated to achieve moderate BCS at

dry-off to cows that had slightly higher BCS at dry-off; the animals with lower BCS had better transition period metabolic (Roche et al., 2015) and immune status (Crookenden et al., 2017).

Independent of ΔBC, lameness during the dry period was associated with transition disease. Chronically lame cows tended to be more likely to develop metritis, and there was an association between proportion of weeks lame during the dry period and metritis development. A similar result was found for TD. These results support our findings that lameness around dry-off was associated with metritis and TD – i.e. cows that were lame around dry-off continued to be lame during the dry period. Using only one lameness assessment within 3 wk before calving, Vergara et al. (2014) reported an increased likelihood for severely lame, but not moderately lame cows to be treated for transition diseases in the post-calving period. The use of cross-sectional data to categorize cows as moderately lame may result in a high proportion of false positives (Eriksson et al.), biasing the estimates towards the null (Dohoo et al., 2012) perhaps explaining the lack of association for moderate lameness reported by Vergara et al. (2014).

Lameness was not independently associated with SCK. Similarly, Kaufman et al. (2016) did not retain lameness in their final model exploring risk factors for SCK. This is not unexpected given that thin cows have lower likelihood of SCK (Vanholder et al., 2015) and are likely to experience repeated cases of lameness (Randall et al., 2015). For this reason, we explored the interplay between lameness and BCS at calving on the occurrence of SCK. By separately analyzing the subgroup of cows that were fat at calving our results suggests that fat cows that are also chronically lame during the dry period are at increased likelihood of developing SCK postpartum compared to sound fat cows. Further studies on SCK and lameness should account for this relation by exploring the interaction between BCS and lameness status.

We evaluated the additive effects of body condition loss during the dry period and the BCS at calving on metritis, SCK and TD. We found that both ΔBC and BCS at calving were independently associated with likelihood of SCK. Cows that are over conditioned around calving have higher risk of SCK (e.g. Vanholder et al., 2015). Although Kaufmann et al. (2016) also reported an effect of ΔBC on the likelihood of SCK, they limited their measures of BCS change from 3 weeks before calving to 2 weeks after calving, which meant that changes in BCS may have been a consequence of SCK.

For metritis and TD, ΔBC (but not BCS at calving) was associated with disease likelihood. Previous studies that have described BCS at calving as a risk factor for metritis (e.g. Duffield et al., 2009) did not included changes in body condition in their models. We speculate that the associations between BCS at calving and metritis described in previous studies may have been a proxy for ΔBC. When studying ΔBC during the dry period, Chebel et al. (2018) also found an association between body condition loss during the dry period and increased likelihood of uterine diseases.

Chronic lameness during the dry period explained some of the variation in the likelihood of transition diseases even after controlling for ΔBC, average feeding time during the close-up period, parity, milk production and BCS at calving. This finding does provide some evidence that lameness may be related to transition diseases through a different pathway than feeding time. Given that lame cows have higher levels of inflammation (Tadich et al., 2013), this could lead to an increased susceptibility to transition diseases (see review by Bradford et al., 2015). Studies on haptoglobin levels, a marker for inflammation, between prepartum sound and lame cows may provide additional insights on the interplay between lameness and transition period diseases.

Based on these findings we believe that preventing lameness cases during the dry period by checking and treating lame cows during the dry-period as well as improving prophylactic treatment for digital dermatitis during the dry period could be useful in reducing lameness cases and lameness case length, resulting in less risk for transition diseases. However, few studies have assessed the efficacy of such lameness treatment practices during the dry period. Furthermore, we suggest more research and efficient ways of controlling BCS from mid-lactation onwards to achieve moderate BCS around dry-off, minimizing the impact of body condition loss on transition health (e.g. Schuh et al., 2019).

4.5 Conclusion

Lameness at dry-off was associated with increased odds of metritis and TD. These results were further supported by our exploratory analyses, showing that chronic lameness during the dry period was associated with higher odds of being sick. One of the mechanisms through which lameness may be associated with transition diseases is through decreased feeding time; throughout the dry period lame cows spent less time feeding than sound cows, and lower feeding time was in turn associated with higher odds of transition diseases. Independently from lameness, body condition loss during the dry period was also associated with increased odds of transition period diseases. Moreover, body condition loss was associated with BCS at dry-off; cows that were fat at dry-off lost body condition while thin cows gain body condition during the dry period. These results suggest that reducing lameness during the dry period, and avoiding over condition at dry-off, may improve transition health.

Chapter 5: General discussion, limitations and conclusions

I began this thesis with a broad review of transition period diseases and the associated cow- and herd-level risk factors. In reviewing the literature, it became apparent that there was a dearth of work on the risk factors for transition period diseases in grazing systems.

The results from Chapter 2 provide some of the first evidence that on some small-scale, year-round, grazing dairy farms located in the south of Brazil the prevalence of transition diseases is similar to that observed in intensive, indoor production systems. Therefore the perception that cows in grazing systems experience better health (Schuppli et al., 2014) was not substantiated by these findings.

A companion study provided further evidence that these small scale farms also had a high prevalence of lameness (Bran et al., 2018). The work described in Chapter 2 was part of a broader research study that included interviews with participating farmers. Through the interviews, it became clear that the farmers believed their cows do not suffer from major diseases because they were low producing, were continuously on pasture, and that the majority of the transition diseases were not applicable in their production system (see Olmos et al. 2018). Perhaps these perceptions explain why preventive protocols for transition period diseases were not in place in the majority of the participating farms. This may have contributed to the high prevalence of transition disease.

Cross sectional studies are weak for drawing causal inferences; however, due to their applicability and simple design they can be used to explore a wider population, capturing a range of practices and conditions. For this reason, we chose to use a cross sectional design for the study reported in Chapter 2. These types of studies describing the current problems faced by the dairy

industry are helpful in informing future research (see examples: Drackley, 1999; Randall et al., 2019).

Subsequent research arising from the findings reported in Chapter 2 is to rank which of the risk factors contribute more for increased disease risk – through for example, investigating the attributable fractions. Nonetheless, future research arising from the findings described in Chapter 2 include studying the effect of restricted water access on welfare, a common occurrence on many farms (Daros et al., 2019), understanding the effect of managing the maternity pen and also the effect of keeping cows and calves together after birth (see Beaver et al., 2019). For example, we found an association between cow-calf contact for at least 12h after birth and reduced incidence of RP. To date most of the RP research has focused on the pre-partum factors that influence the immune system and hormonal balance that increased risk of retained placenta (Laven and Peters, 1996; Mcnaughton and Murray, 2009; Beagley et al., 2010). Nonetheless, the presence of the calf increases circulating oxytocin in the dam (Lupoli et al., 2001), which stimulates uterine contractions (Beagley et al., 2010), providing a possible mechanism for the association between calf presence and placental release. Although one study using external oxytocin failed to support this hypothesis (Stevens and Dinsmore, 1997), the pattern and levels of oxytocin release by the dam may differ when she is being suckled (Lupoli et al., 2001) versus when she is injected with oxytocin.

The opportunity to enrol over 50 dairy herds for the study described in Chapter 2 allowed us to investigate whether there was an association between breed and transition cow disease. We had multiple mixed breed herds in our study, allowing us to also make breed comparisons within herd. Though is likely that some breeds are better suited for grazing systems (Baudracco et al., 2010), our finding of a higher prevalence of SCK in Jersey cows should be viewed with caution,

as breed specific BHB threshold levels are not yet validated. Further research on which breeds are best adapted to grazing systems under different climatic conditions is warranted.

In reviewing the literature (see Chapter 1), I also noticed that there were few studies on lameness and transition period diseases. The work described in Chapters 3 and 4 was intended to fill this gap. Specifically, Chapter 3 shows a high level of lameness incidence during the dry period, providing a rationale for including specific lameness assessments for dry cows in on farm assurance programs (e.g. FARM: NMPF, 2016; ProAction: NFACC-DFC, 2018). Another key finding from Chapter 4 is that changes in BCS over the dry period are associated with BCS at dry-off. This result provides a starting point to discuss the implementation of specific body condition assessments around dry-off, specifically focusing on the presence of fat cows (BCS > 3.5). For example, assurance programs could set a threshold for the proportion of cows above BCS > 3.5. Currently neither ProAction (NFACC-DFC, 2018) or FARM (NMPF, 2016) mention assessing cows for high BCS.

In contrast to cross-sectional study designs, one of the strengths of using longitudinal designs (i.e. Chapters 3 and 4) is the opportunity to explore the causal relationships between the variables recorded. Although exploring these links (see Figure 4.2) does not prove absolute causality (Dohoo et al., 2012), it does allow for stronger inferences than those from cross-sectional studies. The development of the field of causal inferential statistics from observational data championed by Judea Pearl (e.g. Pearl, 2011) will likely become a must-use analysis for drawing causal inferences from observational data; granted the work described in this dissertation is based on more simple statistical analysis than those proposed by Pearl (such as mediation analysis see e.g. VanderWeele, 2011). The data exploration in Chapter 4 highlighted

the causal path connecting lameness to transition period disease through feeding time, independent of body condition loss during the dry period.

Managing cows to achieve good BCS at dry-off and lameness reduction during the dry period should improve transition health and thus increase productivity. Future research should experimentally control BCS around dry-off and assess disease incidences during the transition period (e.g. Roche et al., 2015; Schuh et al., 2019). Recommendations for how to best achieve good BCS around dry-off are also needed. Preventing lameness during the dry period should be a research focus. Despite industry recommendations advocating for hoof trimming before calving, the results of Chapter 3 indicate that this management practice may not fully prevent the development of new lameness cases during the dry period. Research on the prevention of development of claw horn lesions (one of the main causes of lameness Murray et al., 1996) is needed. The work of Newsome et al. (2016) provides a compelling case for prevention of the first claw horn lesions, especially sole ulcers, as these are associated with development of bone protrusions that will likely cause further damage to the sole tissue resulting in lameness.

Lameness may also be caused by infectious lesions, such as digital dermatitis, a treatable malady needing antibiotics not hoof trimming (Potterton et al., 2012; Orsel et al., 2018). One of the strategies to keep infectious hoof lesions under control is the use of routine footbaths (Potterton et al., 2012). Unfortunately, in the work described in Chapter 3, none of the farms used footbaths for the dry cows and thus we were not able to test if there was an association between use of footbaths and incidence of lameness during the dry period. I encourage future work to assess the effectiveness of footbaths for dry cows.

Another limitation in Chapter 3 was the lack of hoof trimming data for enrolled cows in the week of dry-off. Unfortunately, not all enrolled cows were trimmed, which prevented us from precisely estimating the effects of different types of sole lesions on lameness incidence.

In Chapter 4 we argue that the causal link between lameness and transition period diseases may be mediated through reduced feed intake. Although we do not discard other mechanisms, such as the inflammation hypothesis (discussed in Chapter 4), we speculate that lameness only had a minor contribution to the incidence of transition disease. Metabolomic work shows that some markers for transition disease risk can be identified around dry-off (Zhang et al., 2017), suggesting that cows may be predisposed to transition problems even before cessation of lactation. Future work using larger datasets could explore the fraction attributable to each risk factor – i.e. how much each factor contributes to the total risk (or odds) of disease (e.g. Randall et al., 2018).

Only 44% of cows examined in Chapter 4 were not diagnosed with metritis, SCK or treated for RP, hypocalcaemia or DA. Unfortunately we were unable to capture the incidence of mastitis, one of the most common diseases in dairy cattle (Ruegg, 2017). Hence it is likely that in our study population the number of healthy animals was overestimated, and the number of cows suffering from more than one condition may have been high. Borrowing from the medical literature, I encourage future work to investigate the concept of comorbidity (Valderas et al., 2009), which includes not only the concept of having multiple diseases at the same time, but also acknowledges the complexity of the patient, taking into consideration how patients perceive themselves, the environment they are in and how they interact with it. Under some production systems cows may be lacking the resources needed to cope with their needs or be forced to experience a competitive social environment. These sources of environmental stress have been

associated with disease (see review by Proudfoot and Habing, 2015). Broadening our understanding the physiological mechanism predisposing cows to transition diseases may be a starting point (Trevisi and Minuti, 2018), but I believe we will only be able to achieve major improvements in understanding transition health when we incorporate individual differences (see Neave et al., 2017) and how individuals cope with their environment.

When taken together, the findings of the literature review and the results presented in Chapter 4 suggest that there is a myriad of factors that take place far before disease diagnosis and that these may be causally linked to transition health. Perhaps extending the transition period to include the transition from lactating to dry (see Dancy et al., 2019) may provide additional insight. Recent research into changes in behaviour and physiology at dry-off (Dancy et al., 2019), and understanding of mammary gland energy requirements throughout the lactation cycle (see review: Gross and Bruckmaier, 2019), challenge our understanding on the factors affecting the transition cow.

5.1 Conclusions

This thesis contributes to the general understanding of the risk factors for transition period diseases and indicate possible areas of research to improve transition health.

In our study in grazing dairy cows we found a high prevalence of transition period diseases comparable to those reported for dairy cows in intensive indoor systems, suggesting that transition period diseases may also be a major health issue in year-round grazing systems. For retained placenta for example, we found higher levels in grazing cows than in zero-grazing cows. Also, in our study in grazing cows, some management practices, such as type of water access for lactating cows and milking area cleanliness, were identified as risk factors for transition disease.

This indicates that some changes in management in grazing dairy herds may improve transition health. Assuming that grazing cows have better health only because they have pasture access year-round may jeopardize the welfare of these animals.

In indoor housed cattle, we found a high incidence of lameness during the dry period, while cure rates for lameness during the same period were moderate. These results suggest that the dry period may be a risk period for the development of lameness. Moreover, lameness during the dry period is a risk factor for the development of transition disease. We found that lameness reduces feeding time during the weeks prepartum suggesting that lameness may be causally linked to transition disease through reductions in feed intake. We also identified that losing body condition during the dry period, a factor almost exclusive for cows that were fat at dry-off, was associated with increased risk of developing transition disease. Managing cows to avoid over conditioning at dry-off and preventing lameness during the dry period may result in better transition health.

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Appendices

Appendix A List of studies that reported prevalence and/or incidence of transition period diseases by production system type

Study	Study type	Disease	Production system	Prev. (%)	Inc. (%)	n of herds	Total n cows	Year	Country
Dubuc et al., 2010	longitudinal	metritis	indoor	NA	17.6	3	1378	2009	US/CAN
Armengol and Fraile, 2015	experimental	metritis	indoor	NA	29.7	1	1044	2014	Spain
Bicalho et al., 2017	experimental	metritis	indoor	NA	21.7	1	116	NA	US
Pohl et al., 2016	experimental	metritis	indoor	NA	16.7	6	660	2015	GER
Vergara et al., 2014	longitudinal	metritis	indoor	NA	19.4	4	1309	2010	US
Drillich et al., 2001	experimental	metritis	indoor	18.7	NA	1	1756	1999	GER
Vergara et al., 2014	longitudinal	MF	indoor	NA	0.6	4	1309	2010	US
Vergara et al., 2014	longitudinal	RP	indoor	NA	8.9	4	1309	2010	US
Quiroz-Rocha et al., 2009	longitudinal	RP	indoor	NA	15.5	20	1038	1999	CAN
Han and Kim, 2005	longitudinal	RP	indoor	NA	18.7	9	805	2004	Korea
Berge and Vertenten, 2014	longitudinal	SCK	indoor	NA	39	76	2966	2013	EU
Duffield et al., 2009	cross-sectional	SCK	indoor	24.2	NA	25	1010	NA	CAN
Tatone et al., 2017	cross-sectional	SCK	indoor	27	NA	3020	166780	2015	CAN
Vanholder et al., 2015	cross-sectional	SCK	indoor	58.8	NA	23	1715	NA	HOL
Chandler et al., 2018	cross-sectional	SCK	indoor	16	NA	16	1005	2015	US
Dohoo and Martin, 1984	cross-sectional	SCK	indoor	18	NA	32	1234	1981	CAN
Duffield et al., 1997	cross-sectional	SCK	indoor	25	NA	92	1333	1992	CAN
Mahrt et al., 2015	longitudinal	SCK	indoor	10	25	3	305	NA	GER
McArt et al., 2012	longitudinal	SCK	indoor	15	43.2	4	1717	2010	US
Rathbun et al., 2017	longitudinal	SCK	indoor	NA	19.7	1	570	2015	US
Rutherford et al., 2016	cross-sectional	SCK	indoor	17	NA	3	203	2014	ENG
Santschi et al., 2016	cross-sectional	SCK	indoor	22.9	NA	4242	498310	2015	CAN
Süss et al., 2016	cross-sectional	SCK	indoor	10.4	NA	1	240	2015	SLO
Vince et al., 2017	cross-sectional	SCK	indoor	15.8	NA	107	841	NA	Croatia
Joosten et al., 1987	longitudinal	RP	mixed	NA	6.6	NA	312000	1984	HOL

Appendix A, continued.

Berge and Vertenten, 2014	longitudinal	SCK	mixed	NA	46	52	1657	2013	EU
Chapinal et al., 2011	longitudinal	metritis	NA	NA	16.7	55	2365	2007	US
Suthar et al., 2013	longitudinal	metritis	NA	NA	9.6	528	5884	2011	EU
Chapinal et al., 2011	longitudinal	RP	NA	NA	7.4	55	2365	2007	US
Gröhn et al., 1990	longitudinal	RP	NA	NA	4.4	NA	61124	1983	FIN
Suthar et al., 2013	longitudinal	RP	NA	NA	10.4	528	5884	2011	EU
Suthar et al., 2013	cross-sectional	SCK	NA	21.8	NA	528	5884	2011	EU
Giuliodori et al., 2013	longitudinal	metritis	other	39.3	NA	1	303	NA	ARG
Benzaquen et al., 2007	longitudinal	metritis	other	NA	21	1	450	2004	US
Ribeiro et al., 2013	cross-sectional	metritis	pasture	5.3	NA	2	957	NA	US
Sepúlveda-Varas et al., 2015	longitudinal	metritis	pasture	NA	17.3	6	307	2012	Chile
McDougall, 2001	longitudinal	RP	pasture	NA	1.7	11	2652	1999	NZ
Sepúlveda-Varas et al., 2015	longitudinal	RP	pasture	NA	8.8	6	307	2012	Chile
Stevenson, 2000	longitudinal	RP	pasture	NA	3.2	8	1405	1995	AUS
Compton et al., 2014	cross-sectional	SCK	pasture	23.8	NA	57	1620	NA	NZ
Compton et al., 2015	longitudinal	SCK	pasture	16.7	66.5	15	578	NA	NZ
Garro et al., 2014	cross-sectional	SCK	pasture	10.8	NA	1	107	NA	ARG
Ribeiro et al 2013	cross-sectional	SCK	pasture	35.4	NA	2	957	NA	US
Sepúlveda-Varas et al., 2015	longitudinal	SCK	pasture	NA	16.6	6	307	2012	Chile

Appendix B List of potential explanatory variables in chapter 3

List of potential explanatory variables to be included in the models.

B.1 Potential explanatory variables for subclinical ketosis

Potential explanatory variables selected trough unconditional modeling[1] to be offered to multivariable multi-level logistic regression model for subclinical ketosis[2].

Potential explanatory variables	*Value*		Estimates	SE	95% Confidence Interval		P-value
					Lower	Upper	
Breed	*Crossbreed*	Ref					
	Holstein	-	-0.00	0.33	-0.65	0.67	0.99
	Jersey	-	0.63	0.37	-0.10	1.38	0.09
Parity	*1st lactation*	Ref					
	2nd lactation	-	0.37	0.37	-0.35	1.11	0.31
	≥ 3 lactation	-	1.07	0.30	0.49	1.70	<0.001
Body Condition	*<3.0*	Ref					
	3.0 - 3.5	-	0.43	0.24	-0.05	0.92	0.08
	>3.5	-	0.74	0.31	0.13	1.36	0.02
Milk yield (L/d)	*Continuous*	-	0.02	0.02	-0.1	0.06	0.19
DIM	*Continuous*	-	-0.03	0.02	-0.07	0.00	0.09
Metritis	*No*	Ref					
	Yes	-	-0.65	0.38	-1.47	0.07	0.09
Herd size	*Continuous*	-	-0.02	0.01	-0.04	0.01	0.18
Prevalence of down cow	*Low (<5%)*	Ref					
	Medium (5-10%)	-	0.43	0.34	-0.27	1.12	0.21
	High (>10%)	-	1.16	0.33	0.50	1.85	<0.001
Protected fat fed	*No*	Ref					
	Yes	-	0.57	0.38	-0.19	1.36	0.13
Dry period main feed	*Corn silage*	Ref					
	Corn silage and pasture	-	-0.64	0.28	-1.23	-0.06	0.03
Dry period environment	*Extensive grazing*	Ref					
	Feedlot	-	0.63	0.35	-0.7	1.35	0.07
	Rotational grazing	-	0.23	0.37	-0.53	0.97	0.53
Pre-partum Transition period environment	*Extensive grazing*	Ref					
	Feedlot	-	0.72	0.30	0.11	1.35	0.02
	Rotational grazing	-	0.28	0.45	-0.64	1.18	0.53
Access to shade	*Free*	Ref					
	Limited	-	0.56	0.36	-0.14	1.31	0.11
Access to water	*Free*	Ref					
	Limited	-	0.57	0.28	0.00	1.15	0.05

[1] Liberal p-value ≤ 0.2. Herd specified as random effect. [2] Cow was considered subclinical ketotic when blood BHB ≥ 1.2mmol/L.

B.2 Potential explanatory variables for metritis

Potential explanatory variables selected trough unconditional modeling[1] to be offered to multivariable multi-level logistic regression model for metritis[2].

Potential explanatory variables	*Value*		Estimates	SE	95% Confidence Interval Lower	Upper	P-value
Breed	*Crossbreed*	Ref	-2.27	0.37	-3.06	-1.06	<0.001
	Holstein	-	0.30	0.38	-0.41	1.08	0.43
	Jersey	-	-1.14	0.58	-2.37	-0.03	0.05
Body Condition	*<3.0*	Ref	-1.70	0.23	-2.18	-1.28	<0.001
	3.0 - 3.5	-	-0.99	0.29	-1.58	-0.43	<0.001
	>3.5	-	-0.74	0.40	-1.57	0.00	0.06
Milk yield (L/d)	*Continuous*	-	-0.04	0.02	-0.08	0.00	0.06
DIM	*Continuous*	-	0.08	0.03	0.03	0.13	0.002
Dystocia	*No*	Ref	-2.34	0.18	-2.74	-2.02	<0.001
	Yes	-	1.01	0.33	0.34	1.64	<0.01
Retained placenta	*No*	Ref	-3.06	0.25	-3.61	-2.63	<0.001
	Yes	-	2.93	0.32	2.32	3.58	<0.001
Ketosis	*No*	Ref	-2.10	0.19	-2.51	-1.77	<0.001
	Yes	-	-0.68	0.38	-1.49	0.02	<0.08
Herd size	*Continuous*	-	-0.02	0.01	-0.05	0.01	0.18
Dairy cattle health course attendance	*No*	Ref	-1.92	0.21	-2.38	-1.55	<0.001
	Yes	-	-0.62	0.32	-1.28	0.00	0.05
Access to pond	*No*	Ref	-2.40	0.21	-2.87	-2.04	<0.001
	Yes	-	0.68	0.32	0.05	1.34	0.03
Holding area cleanness	*Clean*	Ref	-2.61	0.26	-3.19	-2.15	<0.001
	Dirty	-	0.76	0.31	0.15	1.41	0.02
Prev. (%) of subclinical mastitis	*Continuous*	-	3.64	1.35	0.95	6.39	<0.01
Silage area (ha) per cow	*Continuous*	-	1.64	1.17	-0.70	4.07	0.16
When cow is milked after calving	*Right after*	Ref	-1.96	0.24	-2.48	-1.52	<0.001
	Up to 12h	-	-0.42	0.31	-1.07	0.20	0.18
Calf allowed to suckle colostrum	*No*	Ref	-2.03	0.21	-2.49	-1.66	<0.001
	Yes	-	-0.44	0.33	-1.14	0.21	0.19

[1] Liberal p-value ≤ 0.2. Herd specified as random effect. [2] Metritis score was adapted from Sheldon et al (2006). Cows were considered metritic when between 3 to 14 DIM metritis score = 4 (puerperal metritis). From 15 to 21 DIM cows were considered metritic when scores = 2, 3 (clinical metritis) or 4.

B.3 Potential explanatory variables for retained placenta

Potential explanatory variables selected trough unconditional modeling[1] to be offered to multivariable multi-level logistic regression model for retained placenta.

Potential explanatory variables	*Value*		Estimates	SE	95% Confidence Interval Lower	Upper	P-value
Breed	*Crossbreed*	Ref					
	Holstein	-	0.70	0.36	0.03	1.46	0.05
	Jersey	-	-1.58	0.68	-3.11	-0.36	0.02
Body Condition	*<3.0*	Ref	-1.57				
	3.0 - 3.5	-	-0.49	0.25	-0.99	0.00	0.05
	>3.5	-	-0.37	0.35	-1.09	0.28	0.28
Parity	*1st lactation*	Ref					
	2nd lactation	-	0.37	0.39	-0.40	1.16	0.35
	≥ 3 lactation	-	0.75	0.33	0.14	1.43	0.02
Dystocia	*No*	Ref					
	Yes	-	1.18	0.29	0.60	1.74	<0.001
Herd size	*Continuous*	-	-0.02	0.01	-0.04	0.00	0.13
Use of maternity pen	*No*	Ref					
	Yes	-	-0.41	0.32	-1.08	0.20	0.20
When fresh cow is regrouped	*Up to 12h*	Ref					
	More than 12h	-	-0.55	0.25	-1.09	-0.06	0.03
Time of cow-calf separation	*Up to 12h*	Ref					
	More than 12h	-	-0.76	0.32	-1.44	-0.16	0.02
Calf allowed to suckle colostrum	*No*	Ref					
	Yes	-	-0.49	0.26	-1.02	0.01	0.06
Pre-partum Transition period environment	*Extensive grazing*	Ref					
	Feedlot	-	-0.26	0.27	-0.82	0.28	0.35
	Rotational grazing	-	-0.72	0.47	-1.74	0.14	0.12

[1] Liberal p-value ≤ 0.2. Herd specified as random effect.