MANAGEMENT PRACTICES AND COW-LEVEL FACTORS RELATED TO CLAW HORN DISRUPTION LESIONS IN DAIRY COWS

by

HANNA KRISTINA ERIKSSON

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

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Examining Committee:

Daniel M Weary
Supervisor

Marina AG von Keyserlingk
Supervisory Committee Member

Jonathan N Huxley
External Examiner

David D Kitts
University Examiner

Anthony P Farrell
University Examiner

Additional Supervisory Committee Members:

Ronaldo LA Cerri
Supervisory Committee Member

Frances S Chen
Supervisory Committee Member

Abstract

Prolonged standing has been related to an increased risk of claw horn disruption lesions (CHDL), likely by increasing the mechanical load on the hoof. Standing behaviour can be affected by management practices. For example, prolonged standing has been reported for primiparous animals after mixing with older cows, presumably as a result of increased social competition. The hoof is believed to be more vulnerable to mechanical damage around calving, making this a period of a particular interest. The aims of this thesis were to evaluate the relationship between standing behaviour around calving and CHDL later in lactation, and to investigate if the social environment affects how heifers behaviourally react to regrouping. In Chapter 1, I summarise the current knowledge regarding factors that can affect standing behaviour, with particular focus on the social environment. As there is no consensus for how to interpret longitudinal locomotion data, I evaluate how assessment frequency, and lameness definition affect measures of lameness incidence in Chapter 2. A single observation of compromised locomotion as criterion for lameness was poorly related to more strict lameness definitions, and to the presence of claw lesions at trimming. In Chapter 3, I evaluate if the positive association between prolonged standing and sole lesions found in experimental studies is present also on commercial farms. Standing time and standing bout duration during the first 2 wk after calving were positively related to the odds of developing sole lesions later in lactation, as was an increase in standing bout duration from pre- to postpartum. In Chapter 4, I investigate if the social environment influence the standing behaviour of newly calved heifers after regrouping. I found no difference in standing behaviour between heifers regrouped to a low-stocked pen with familiar animals and heifers mixed with multiparous older cow at 100%

stocking density, and under the conditions used in this study regrouping did not cause prolonged standing. These studies provide evidence that standing behaviour during the transition period is temporally related with CHDL on commercial farms, and suggest that the effect of regrouping on standing behaviour is influenced by the conditions under which regrouping occurs.

Lay Summary

Long standing time has been associated with mechanical damage to the hoof. Around calving the hoof may be particularly sensitive to this type of damage, so it is possible that prolonged standing time during this period is particularly detrimental. Previous research has found that young cows often spend long times standing after they are mixed with older cows. The goals of this thesis were to evaluate if cows with long standing time around calving were more likely to have injured hooves, and to investigate if young cows housed in a low-stress social environment after regrouping spent less time standing than heifers mixed with older cows. I found that cows that spent longer time standing directly after calving were more likely to have damaged hooves 2 – 3 month afterwards, but did not confirm that the social environment affect standing behaviour after regrouping.

Preface

For the work presented in Chapter 2 H. K. Eriksson designed the study, collected data, performed the statistical analyses and wrote the manuscript. R. R. Daros provided input during the development of the project, helped with data collection and statistical analysis, and gave feedback on manuscript drafts. D. M. Weary and M. A. G. von Keyserlingk acted in the typical role of supervisors, helping with statistical analysis, and providing input and editing drafts. This project received UBC Animal Care approval (certificate number A15-0084).

For the work presented in Chapter 3 H. K. Eriksson developed the main ideas, designed the study, collected data, performed the statistical analyses and wrote the manuscript. R. R. Daros provided input during the development of the project, helped with data collection and statistical analysis, and gave feedback on manuscript drafts. D. M. Weary and M. A. G. von Keyserlingk acted in the typical role of supervisors, giving feedback during project development and data collection, helping with statistical analysis, and providing input and editing drafts. This project received UBC Animal Care approval (certificate number A15-0084).

For the work presented in Chapter 4 H. K. Eriksson developed the main ideas, designed the study, collected all the data, performed the statistical analyses and wrote the manuscript. D. M. Weary and M. A. G. von Keyserlingk acted in the typical role of supervisors, giving feedback during project development and data collection, helping with statistical analysis, and providing input and editing drafts. This project received UBC Animal Care approval (certificate number A14-0040).

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List of Abbreviations

AMS = automatic milking system

BCS = body condition score

BI = bias index

CHDL = claw horn disruption lesion

GS = gait score

IQR = interquartile range

OR = odds ratio

PI = prevalence index

RH = relative humidity

SD = standard deviation

THI = temperature-humidity index

Glossary

305-d milk yield	a standardised way of calculating the farm's average lactation milk yield/cow
actor event	butt/push from the focal cow immediately results in complete withdrawal of the other cow's head from under the feed barrier
ASSM1 to ASSM3	assessment frequencies evaluated in Chapter 2; from weekly locomotion data collected during the dry period, every (ASSM1), every other (ASSM2), or every third (ASSM3) assessment was retained in the data set, and the animals were categorised as remaining sound, or becoming lame based on the available information
bias index	the extent to which observers disagree on the proportion of cases. The index can theoretically take a value between -1 (observer A considers all subjects to be a case, while observer B considers no subject to have the outcome) and 1 (the opposite situation) with 0 indicating no systematic difference in scoring between the observers
CON	control treatment in Chapter 4; newly calved heifers were housed in fully stocked pens and mixed with multiparous animals for 3 wk after calving
convection	the process by which heat energy is transferred by movement of a heated fluid such as air or water
corium	the horn-producing cells in the epidermis of the hoof
dry-off	the end of the lactation, most commonly approximately 60 d before the next calving
dry period	the time period between dry-off and the next calving, when the cows do not produce milk
dyad	the relationship between two animals
head-lock	a self-locking restraining device at the feed barrier
heifer	female cattle before first calving
LAME1 to LAME3	lameness definitions evaluated in Chapter 2; lameness were defined either as a gait score of 3 or higher observed at least once (LAME1), as at least two consecutive gait scores of 3 or at least one gait score of 4 (LAME2), or as at least

	three consecutive gait scores of 3 or at least one gait score of 4 (LAME3).
lameness	a modification of the gait in response to pain in the leg or hoof
LS	low-stocked treatment in Chapter 4; newly calved heifers were housed in a low-stocked pen with familiar conspecifics of the same age for 3 wk after calving
multiparous	dairy cow that has calved more than one time
perching	standing with the front feet in the free-stall
post-and-rail feed barrier	open type of feed barrier delimited by a horizontal neck rail
prevalence index	index measuring the how much the proportion of agreement differs between the positive and the negative outcome. The index can theoretically take a value between -1 (both observers think that all subjects have a negative outcome) and 1 (both observers think that all subjects have a positive outcome) with 0 indicating that there is no difference in the proportion of agreement between the positive and the negative outcome
primiparous	dairy cow that has calved one time
radiation	the emission of heat energy as electromagnetic waves
reactor event	butt/push from another cow immediately results in complete withdrawal of the focal cow’s head from under the feed barrier
sound	gait not associated with lameness; generally the term indicates that the animal is not limping
stocking density	the relative number of cows per unit of resource; a free-stall stocking density of 100% means that there is one stall available for each cow in the pen
thermal conductivity	the ability of a material to transfer heat
transition period	most commonly defined as the period from 3 wk before to 3 wk after calving

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For E and others like her

Chapter 1: Introduction

1.1 Background

Lameness is one of the greatest welfare challenges for the dairy cow. Despite decades of research the disorder remains common (reviewed by Archer et al., 2010; Bicalho and Oikonomou, 2013), although recent research suggests that the prevalence of lameness may currently be decreasing in UK (Randall et al., 2019). On average, one of every 4 – 6 lactating cows is lame in Canada (von Keyserlingk et al., 2012; Solano et al., 2015; Westin et al., 2016), with prevalence above 70% on some farms (e.g. von Keyserlingk et al., 2012). These numbers are similar to what has been found in other countries with intensive production systems (UK; Randall et al., 2019; US; Chapinal et al., 2013; the Netherlands; de Vries et al., 2015). In addition to the negative effects on welfare, lameness is also a costly disorder; decreased milk production (Amory et al., 2008), reduced fertility (Hernandez et al., 2001), and increased culling risk (Booth et al., 2004) have been related to lameness.

The majority of lameness cases are caused by lesions in the claws (Offer et al., 2000; DeFrain et al., 2013). Claw lesions have broadly been classified into infectious and non-infectious (Mason, 2009), with different risk factors identified for the two types. Although infectious claw lesions are painful, and unquestionably affect the welfare of dairy cattle (Whay et al., 1998), the focus of this thesis is non-infectious claw horn lesions.

Claw horn disruption lesions (CHDL; sole haemorrhages, sole ulcers and white line lesions) are among the most common causes for lameness (Murray et al., 1996; Green et al., 2014). Minor lesions, seen as yellow discoloration or mild bleedings on the sole surface, usually do not impair locomotion (Whay et al., 1997), but more severe lesions, such as sole ulcers, can cause severe lameness (Whay et al., 1998; Sogstad et al., 2005; Flower and Weary, 2006).

Lesions deep enough to expose the underlying soft tissue can induce hypersensitization to a blunt pin for more than 28 days, indicating that CHDL can cause chronic pain (Whay et al., 1998). Being diagnosed with severe CHDL at the end of the lactation increases the risk of having claw horn lesions also in the subsequent lactation (Manchado et al., 2011), which suggests that CHDL can cause long-term damage to the claw. The importance of early detection is further stressed by a substantially higher response rate to treatment in acutely lame cows diagnosed with CHDL, compared to treatment of chronically lame animals (Thomas et al., 2015, 2016).

Previously, much research focused on how the housing environment and management affect claw health (e.g. Leonard et al., 1996; Vanegas et al., 2006; Telezhenko et al., 2009). Although epidemiological studies have provided insight into environmental risk factors (e.g. Somers et al., 2003; Chapinal et al., 2013), experimental studies have often been poorly replicated (Livesey et al., 1998; Webster, 2002; Leach et al., 2005), making it difficult to draw strong inferences about causation. The prevalence of CHDL (Sogstad et al., 2005) and lameness (Cramer et al., 2008; von Keyserlingk et al., 2012) varies greatly between farms with similar housing systems, and despite much effort to improve housing environment the prevalence of lameness has generally not decreased. Taken together these results suggest that it is important to not only consider the physical environment per se, but also how the animals interact with their environment. For example, standing time has been found to be positively correlated to CHDL (Singh et al., 1993; Galindo and Broom, 2000) and lameness (Galindo and Broom, 2000). Since socially subordinate animals are more likely to change their behaviour when housed in a competitive environment (e.g. Fregonesi et al., 2007a; Proudfoot et al., 2009), it is conceivable that social factors may affect the risk of CHDL, but this has so far received relatively little scientific attention.

In the following sections of this chapter I review the literature, and present identified gaps in our understanding of how behaviour relate to CHDL and lameness. The findings of this review helped guide the empirical work that is presented in Chapter 2 to 4. The objectives and research questions are presented in the end of Chapter 1.

1.2 Development of claw horn disruption lesions

CHDL is predominantly non-severe in heifers, but the number and severity of lesions increases shortly after first calving (Leach et al., 1997; Offer et al., 2000; Webster, 2002). Based on findings from anatomical studies, CHDL is believed to develop when mechanical pressure damages the soft tissue of the claw, causing leakage of serum or blood from the capillary bed (Ossent and Lischer, 1998; Lischer et al., 2002). The disruption of normal circulation reduces the nutrition supply to the horn-producing cells in the epidermis (the corium), leading to synthesis of low-quality horn, into which the extravascular serum and blood are incorporated (Kempson and Logue, 1993; Ossent and Lischer, 1998). In severe cases total disruption of horn synthesis can occur (Ossent and Lischer, 1998), causing severe horn lesions such as double soles or sole ulcers. As the horn grows the lesions become visible on the surface of the sole 6 – 12 wk later (Kempson and Logue, 1993; Livesey et al., 1998; Ossent and Lischer, 1998). The most severe haemorrhages are generally found on the sole (Boosman et al., 1989), and animals with sole ulcers generally have severe circulatory disturbances in the sole segment of the corium (Mochizuki et al., 1996). Indirect support for the role of mechanical pressure on the development of CHDL comes from the fact that severe sole haemorrhages and sole ulcers are most common in the lateral hind claw (Leach et al., 1998; Tsuka et al., 2012), which carries a larger weight load than the other claws (van der Tol et al., 2002, 2003; Meyer et al., 2007).

1.2.1 The protective structures of the claw

The claw has multiple anatomical structures that help mitigate the mechanical stress imposed on the corium when the animal is standing or walking; the most important are 1) the claw capsule, 2) the suspensory apparatus, and 3) the digital cushion.

The claw capsule provides a physical barrier protecting the underlying, more sensitive, structures against injury. The mechanical properties of the horn differ within the capsule; the wall horn is hard and resistant to mechanical deformation, while the sole and especially the heel segment of the claw (the heel bulb) are softer and more elastic (Hinterhofer et al., 2005; Franck et al., 2006). Results from a simulation study suggest that this conformation helps to distribute most of the weight load to the bulb and claw wall, while reducing the mechanical stress on the solar corium (Hinterhofer et al., 2006). Even though the results in this study were based on computer simulations using only one healthy claw specimen, force plate measurements on live animals also show that more weight is carried by the ball and the sole closest to the abaxial wall when the animal is standing (van der Tol et al., 2002) and walking (van der Tol et al., 2003). The hardness of the sole and wall horn, and hence its resistance against wear, depend on the number and diameter of horn tubules, with horn containing a large number of thin horn tubule being harder (Franck et al., 2006). Sanders et al. (2009) reported that thin soles were a common finding in lame animals, and suggested that the risk of developing other types of claw horn lesions was increased for animals with thin soles. The white line is the junction between the sole and the wall horn, and consists of horn with lower mechanical strength. The softer horn makes this area more susceptible to mechanical damage than the rest of the capsule (Budras et al., 1996; Winkler and Margerison, 2012).

The suspensory apparatus consists of tense fibrous threads that anchor the pedal bone to the interior surface of the claw wall; this creates a narrow space between the ventral surface of pedal bone and the sole that is occupied by the corium and the digital cushion (Lischer et al., 2002). Compared to horses, the suspensory apparatus of cattle is less well developed (Lischer et al., 2002), and the tensile strength is lower in the axial than in the abaxial segment of the apparatus (Maierl et al., 2002). Lischer et al. (2002) suggested that this asymmetry in biomechanical strength causes the pedal bone to tilt during the stride, thereby increasing the focal pressure at the axial part of the sole-bulb junction, corresponding to the site where sole ulcers most commonly develop. A recent case study used multiple radio-opaque markers inserted into the skeleton of a limb specimen to assess the three-dimensional changes in the spatial configuration of the bone structures during weight load (Ouweltjes et al., 2016). The results provide some evidence that the pedal bone rotates during weight load, but caution is warranted when making inferences since only one abattoir specimen was evaluated.

In a study using post-mortem specimens from animals with known age, parity and lactation stage, Lischer et al. (2002) reported that the distance between the pedal bone and the inner surface of the sole was smaller in animals with sole ulcers compared to healthy controls. Histological assessment of the suspensory apparatus ruled out that this reduction in distance was caused by rupture of the fibrous treads connecting the pedal bone to the claw wall, which had been the predominant theory prior to this study. Tarlton et al. (2002) instead proposed that the increase in relaxin and oestrogen around partus increases the laxity of the suspensory apparatus, leading to an increase in mechanical pressure on the sole during weight load. Although hormone levels were not measured in the latter study, the group found that the tensile strength of the suspensory apparatus in primiparous cows was reduced for at least 12 wk postpartum.

Comparing age-matched maiden heifers with newly calved primiparous cows, Knott et al. (2007) verified this result, and also found more severe CHDL in the primiparous cows than in their identically housed and fed maiden counterparts. These results support the idea that increased laxity in the suspensory apparatus around calving constitutes an important risk factor for impaired claw health. In a recent prospective longitudinal study Newsome et al. (2017a) reported that the shortest distance between the inner surface of sole and the ventral part of the pedal bone occurred during the first week postpartum. In a follow-up study, insulin resistance in early lactation was suggested as a possible cause for the increased laxity in the suspensory apparatus after calving (Newsome et al., 2017b).

Since the suspensory apparatus is less well developed in cattle, this species relies to a higher degree on the digital cushion to mitigate the negative effects of weight load (Lischer et al., 2002). The digital cushion consists of three connective tissue pads containing soft fat, and is situated between the claw capsule and the ventral part of pedal bone. The cushion covers a large part of the sole, with the thickest part in the ball region. It has been suggested that this anatomical conformation helps to dissipate forces arising within the claw capsule during the heel strike of the stride, and so protects the corium from mechanical damage (Räber et al., 2004). In a cross-sectional study, Bicalho et al. (2009) found that the distance between the inner surface of the sole and the pedal bone (the space occupied by the corium and the digital cushion), as measured by ultrasonography, depended on what month of lactation the individual animal was measured. In newly calved animals the distance was relatively large, while it progressively shortened for animals in their 2nd to 4th month of lactation. For animals measured in later stages of lactation the distance between the sole and the pedal bone again was larger, and it did not differ between cows measured at dry-off and newly calved animals. A shorter distance was generally found in animals with CHDL than in animals without horn lesions, and the distance was strongly and positively related to the body condition score (BCS) of the animals (Bicalho et al., 2009). While acknowledging that the study design made it hard to draw strong inferences, the authors suggested that the functionality of the digital cushion is negatively affected in animals with low BCS. A subsequent longitudinal study reported that animals with thin digital cushion at dry-off had a higher incidence of CHDL during the subsequent lactation (Manchado et al., 2011), providing stronger support for this theory. Additional support, albeit indirect, comes from a longitudinal study exploring the temporal association between BCS and lameness treatment (Green et al., 2014). Lame cows treated for CHDL were more likely to have lower BCS in the month preceding treatment, compared to cows that were not diagnosed as lame. This association was not present for infectious claw lesions, which suggests that low BCS is a risk factor specifically for CHDL. In a prospective study Newsome et al. (2017b) found additional effects of back fat thickness (continuous measure of BCS) and shorter distance between the inner surface of the sole and the pedal bone (indicative of thinner digital cushion) on the risk of future CHDL, which may indicate that these variables at least partly act through independent mechanisms.

It is possible that age can influence the protective capacity of the digital cushion. Räber et al. (2006) found a lower content of soft fat in the digital cushion of heifers, while Bicalho et al. (2009) report thinner cushions in primiparous cows. Together these studies indicate that the functionality of the cushion is not fully developed at first calving at two years of age. Although Räber et al. (2004) found that cows in parity 4 or higher had a smaller amount of fat and more connective tissue in the digital cushion compared to younger animals, Lischer et al. (2002) cited a German dissertation that claims that these properties were not found in older animals with healthy claws. The increase of connective tissue may in part be explained by the commonly occurring formation of new bone on the caudoventral part of the pedal bone (Newsome et al., 2016), situated directly above the digital cushion. It is plausible that the bone growth increases the mechanical pressure on the cushion during standing and walking, which could lead to recurrent trauma and scar formation. Bone growth on the pedal bone has been measured in a large study using abattoir specimens (Tsuka et al., 2012). Although age explained most of the variation in bone growth, animals with sole ulcers had the most extensive bone formations. Similar findings have been reported in a study using claw specimens from animals with known claw lesion status from first calving until culling (Newsome et al., 2016). In the latter study larger bone formations were found in animals that had been diagnosed with CHDL at any point after first calving; this relation was not seen in animals with infectious claw lesions. It is possible that inflammation due to mechanical damage stimulates bone growth, which leads to larger exostosis formation and increased risk of recurrent damage to the corium. This could partly explain the high recurrence rate for CHDL (Manchado et al., 2011), and the unsatisfactory recovery rate after treatment of chronic CHDL-related lameness (Thomas et al., 2016).

Although much is still unknown about the causal mechanism for CHDL development, findings from longitudinal studies provides reasonable evidence that the functionality of the digital cushion and suspensory apparatus varies over the lifetime of the animal. This make it plausible that the risk of developing CHDL are increased during certain periods, such as the transition and early lactation period, during which animals would benefit from extra care.

1.3 Standing behaviour in relation to CHDL and lameness

As the mechanical load on the claws is higher during weight bearing, it is possible that prolonged standing time, here defined as the total time not lying, increases the risk of CHDL. To explore what is currently known about standing behaviour in relation to CHDL development and lameness, longitudinal studies were identified using searches on Web of Science and Google Scholar, and the search terms ‘dairy’ and ‘cow’ in combination with ‘lameness’ or ‘claw lesion’, and ‘standing’ or ‘behaviour’. From the reference list of the identified studies additional publications were identified. As none of the identified studies assessed behaviours in tie-stall housed animals, the evaluation is restricted to loose-house systems. Of 16 identified studies (see Table 1.1. on page 10), the measured behaviours were confounded with treatment in five studies (Leonard et al., 1994; Chaplin et al., 2000; Leach et al., 2005; Olmos et al., 2009; O’Driscoll et al., 2010), one study was poorly replicated (Colam-Ainworth et al., 1989), and one study recorded standing time with an inexact method (O’Connell et al., 2008); these studies were not considered further.

Six of the nine remaining studies reported a positive correlation between increased standing time and either the severity of CHDL (Singh et al., 1993; Leonard et al., 1996; Galindo and Broom, 2000; Chapinal et al., 2009b; Proudfoot et al., 2010) or the risk of lameness (Galindo and Broom, 2000; Galindo et al., 2000). Chapinal et al. (2009b) and Proudfoot et al. (2010) reported longer standing time around calving for animals that developed severe CHDL during the subsequent lactation, indicating a temporal association aligning with the known lag period of 6 – 12 wk between damage to the corium and visible lesions on the sole surface (Livesey et al., 1998). The relation is complex and involves an interaction between standing time and standing surface; e.g. Hernandez-Mendo et al. (2007) found that compared to lame cows continuously housed indoors, lame animals kept on pasture improved their gait despite spending longer time standing. It is possible that the more yielding properties of soil compared to concrete facilitated recovery from lameness. Reduced mechanical stress on the corium could be a contributing factor

Table 1.1 Longitudinal studies relating standing behaviour to CHDL and lameness
The studies were identified in Web of Science and Google Scholar, using combinations of the search terms 'dairy', 'cow', 'lameness', 'claw lesion', 'standing' and 'behaviour', and by scanning the reference lists of identified publications for additional articles.

	Assessed behaviours			Outcomes		
Study type	Standing time	Standing bout	Perching	Lameness	CHDL	References
Case report	x				x	Colam-Ainworth et al., 1989
Prospective cohort	x				x	Singh et al., 1993
Experimental	x		x		x	Leonard et al., 1994
Experimental	x		x	x	x	Leonard et al., 1996
Experimental	x		x	x	x	Chaplin et al., 2000
Prospective cohort	x		x	x	x	Galindo and Broom, 2000
Prospective cohort	x		x	x	x	Galindo et al., 2000
Experimental	x			x	x	Leach et al., 2005
Experimental	x				x	O'Connell et al., 2008
Crossover	x		x	x	x	Bernardi et al., 2009
Prospective cohort	x			x	x	Chapinal et al., 2009b
Experimental	x	x		x	x	Olmos et al., 2009
Experimental	x			x	x	O'Driscoll et al., 2010
Case-control	x	x	x		x	Proudfoot et al., 2010
Experimental	x		x		x	Dippel et al., 2011
Experimental	x	x		x	x	Ouweltjes et al., 2011

for the lower occurrence of CHDL (Webster, 2001; Somers et al., 2003) and lameness (Haskell et al., 2006; Vanegas et al., 2006; Lobeck et al., 2011; Chapinal et al., 2013) generally found in animals housed on soft surfaces; although Fjeldaas et al. (2011) found a higher prevalence of sole ulcers in farms with rubber flooring.

Increased time standing with the front claws in the stall (perching) has also been associated with increased risk of CHDL (Proudfoot et al., 2010; Dippel et al., 2011) and lameness (Galindo and Broom, 2000; Galindo et al., 2000; Bernardi et al., 2009). Proudfoot et al. (2010) reported an increase in standing time due to increased time perching and so could not discern the effect of perching from that of prolonged standing on CHDL risk. However, when Bernardi et al. (2009) experimentally manipulated the cows' ability to stand fully in the stall by placing the neck-rail closer to the back curb, animals spent more time perching without a concurrent increase in standing time. As the majority of new lameness cases occurred during the period with increased perching, this provides some evidence that perching could be related to lameness through a different mechanism than prolonged standing. Using within cow measurements, Chapinal et al. (2009a) concluded that the weight load on the hind legs does not increase when the animals stand with elevated front feet. A more likely, albeit unproven, causal pathway discussed by Dippel et al. (2011) is that perching increases the exposure to manure for the hind claws. Exposure to manure is believed to increase the moisture content and reduce the hardness of the claw horn. Borderas et al. (2004) showed a negative correlation between horn hardness and severity of claw lesions. Together these results could in part explain why more severe CHDL is generally found in the hind claws (e.g., Vermunt and Greenough, 1996).

The Canadian dairy industry standards recommend providing stalls comfortable enough to allow for at least 12 h of daily rest (DCF-NFACC, 2009). This recommendation aligns with the available scientific evidence for indoor housed cattle; for example Proudfoot et al. (2010) found that cows that developed severe sole lesions 7 – 15 wk postpartum stood on average 14 h/d around calving, compared to 12 h/d for animals that maintained good claw health. However, considering that daily lying times ≥12 h is uncommon for dairy cattle kept on pasture (Hernandez-Mendo et al., 2007), when their behaviours are less restricted than in indoor systems, achieving a daily resting time of ≥12 h for all animal through improved stall comfort may be infeasible.

It is possible that individual long standing bouts, especially if performed on a hard surface, also could damage the corium. So far standing bout duration has received little attention as a risk factor for CHDL and lameness; only two of the nine studies discussed above evaluated how standing bout duration relate to claw health. Proudfoot et al. (2010) found longer average standing bouts around calving in animals that developed severe CHDL, compared to animals that continued to have healthy claws. In contrast, Ouweltjes et al. (2011) did not find any effect on claw health when artificially inducing long standing bouts by blocking off access to the lying area during parts of the day. Unfortunately, neither study report maximum standing bout length. The relationship between this aspect of standing behaviour and claw health should be explored further. If individual long standing bouts prove to be an important risk for lameness, it may not be sufficient to just focus on the environment in the home-pen when studying risks factors associated with lameness. In a cross-sectional study Espejo and Endres (2007) found an association between the pen prevalence of lameness and daily time spent milking, suggesting that future lameness work should include all areas of the cows' environment.

1.4 Factors that influence standing behaviour

Multiple recent large-scale epidemiological studies have reported average herd lying times lower than 12 h/d for more than half of the assessed herds (von Keyserlingk et al., 2012; Charlton et al., 2014; Ito et al., 2014), suggesting that many animals spend a considerable amount of time standing. The next section critically reviews the literature describing factors that affect standing behaviour in dairy cows. When it is appropriate the inverse of standing time (i.e. time spent lying) will be reported.

1.4.1 Environmental factors

Depending on the type of housing system (e.g. free- and tie-stall systems, or open packs), and the type and amount of bedding, dairy cows will generally stand for 11 – 15 h/d (Endres and Barberg, 2007; Rushen et al., 2007; Fregonesi et al., 2009b; Abade et al., 2015). This variability in standing time suggests that the comfort of the lying area may influence the risk of CHDL.

1.4.1.1 Lying surface

Hard lying surfaces reduce lying time (concrete: 10.42 h/d vs. mattress 12.25 h/d; Haley et al., 2001; concrete: 8.13 h/d vs. rubber mat: 9.37 h/d; Rushen et al., 2007), while lying time generally increases if ample amount of soft bedding material, such as sawdust (0 kg/stall: 12.3 h/d vs. 7.5 kg/stall: 13.8 h/d; Tucker and Weary, 2004) or straw (1 kg/stall: 11.2 h/d vs. 7 kg/stall: 12.4 h/d; Tucker et al., 2009) covers the lying surface. Similarly, Drissler et al. (2005) reported an increase in lying time with increasing bedding depth in sand-bedded stalls. Tucker et al. (2009) found that the amount of bedding must be enough to increase the compressibility of the material (i.e. increase the softness of surface) to prolong lying time; provision of straw insufficient to cover the lying surface (0.5 – 3 kg/stall) gave no beneficial effect.

Preference studies can tell us much about what aspects of stall design the animals regard as important. Typically these studies start with a period of forced exposure (a 'no-choice' phase) to the different treatments (e.g. types of bedding), to let the animals gain some experience of each of the alternatives. After this period, animals are free to choose between two or more alternatives (the 'choice' phase). In work concerning lying behaviour, the proportion of time animals spend lying in a certain alternative have most often been used as indication for which treatment the animals prefer. Not surprisingly, Tucker and Weary (2004) reported that dairy cows strongly prefer well-bedded stalls (>80% of lying time spent in well-bedded stalls for all tested animals).

The type of bedding material also seems to be important, as sand has generally been found to be less preferred than sawdust (Tucker et al., 2003) and straw (Manninen et al., 2002). In addition, while animals previously exposed to organic bedding maintained a clear preference for this alternative after the no-choice phase, animals with previous experience to sand seemed to find both alternatives equally attractive (Tucker et al., 2003; Norring et al., 2008). While following individual animals for ≥21 wk, Norring et al. (2008) found that animals housed in pens with sand-bedded stalls had lower lying time than cows with access to straw stalls (11.3 and 12.5 h/d respectively).

The amount of bedding in the stalls decreases over time, as bedding is pushed backwards from animal movements or is removed during daily stall maintenance. In sand-bedded stalls the decrease in bedding depth is greatest is in the centre of the stall. An experimental study artificially manipulating the concavity of the sand surface found that daily lying time on average was 1.15 h longer when the stall surface was level with, compared to 6.2 cm below the rear curb at the deepest point (Drissler et al., 2005). Dairy cows also reduce the time they spend lying with increasing moisture content of the bedding (Fregonesi et al., 2007b; Reich et al., 2010), and showed a strong preference for dry stalls (wet stalls: 0.9 h/d vs. dry stalls: 12.5 h/d, with 50% of the animals never lying down in wet stalls; Fregonesi et al., 2007b).

1.4.1.2 Stall design

To avoid manure soiling it is common to equip the lying area with stalls that guide the animals to lie down with their rear end close to the rear curb. The stalls usually also have neck-rails that encourage the animals to back out of the stall shortly after standing up, to reduce the risk of stall soiling. As stall partitions (see Figure 1.1 on page 16) restrict the movements of the animals when they transition between lying and standing it is plausible that stall dimensions can affect lying behaviour.

This idea has been tested by comparing the behaviour of cows in identical test pens where the lying area was either configured to contain free-stalls or altered to provide the animals with an open pack (Fregonesi et al., 2009b), or open stalls divided only with wooden boards protruding slightly over the bedding surface (Abade et al., 2015). During the no-choice phase equal or modestly increased lying time was found for the open solutions (conventional stall: 12.48 h/d vs. open pack: 13.03 h/d; Fregonesi et al., 2009b; conventional stall: 13.2 h/d vs. open stall: 12.9 h/d; Abade et al. 2015). However, when the animals could freely choose between conventional stalls and an unrestricted lying surface, the animals preferred to lie on the open pack (Fregonesi et al., 2009b), while the reversed was found when the open lying area was sectioned with wooden boards (Abade et al., 2015).

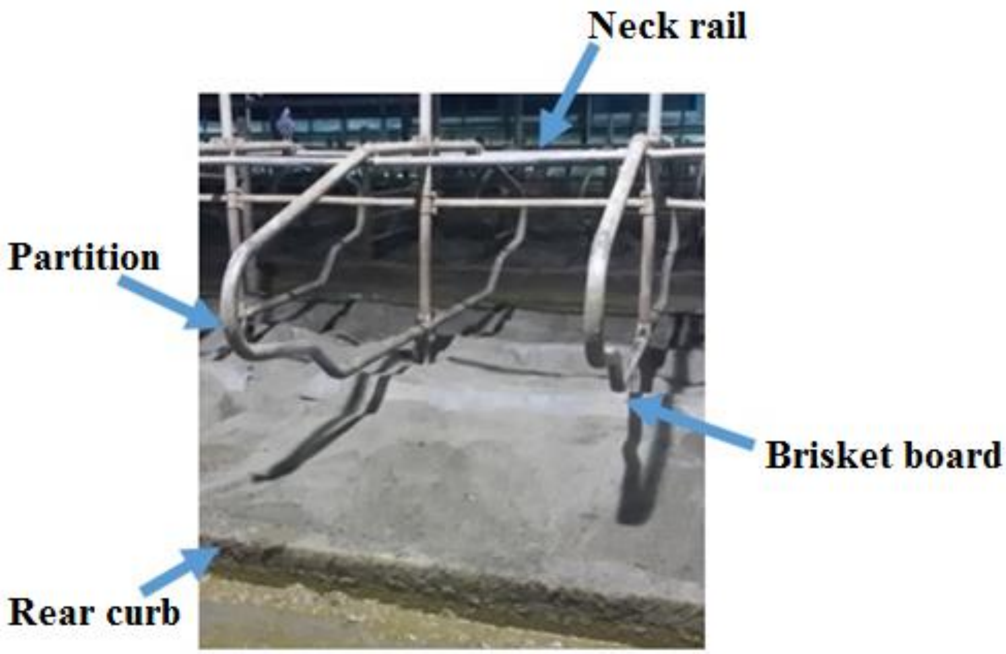

Figure 1.1 Schematic of free-stall design
Arrows illustrates stall parts reported to affect standing time in the peer-reviewed literature.

Other preference work has tested additional stall parts; these studies found no effect on lying time during the no-choice phase when comparing different types of stall partitions (Ruud and Bøe, 2011) and neck-rail placements (Tucker et al., 2005; Bernardi et al., 2009; Fregonesi et al., 2009a), while the presence of a brisket board reduced lying time (brisket board: 11.6 h/d vs. no brisket board: 12.8 h/d; Tucker et al. 2006). Longer animals were more likely than short cows to lie more forward in the stalls lacking a brisket board, suggesting that the animals tried to avoid physical contact with the rear curb when possible. Tucker (2004) found longer lying times when animals were provided wide compared to narrow free-stalls (wide stalls: 10.8 h/d vs. narrow stalls: 9.6 h/d); however, during the choice phase the animals did not show any clear preference for the wider stalls. Likewise, neck-rail placement appears to not influence which stall dairy cows choose to lie down in (Tucker et al., 2005).

Together these results suggest that even if normal lying times can be achieved in well maintained free-stalls, dairy cows find physical contact with stall parts aversive and prefer an unrestricted lying surface. This could explain why animals given both indoor and outdoor access generally used the pasture as the preferred lying surface (Falk et al., 2012). When choosing a lying area the animals seem to direct more attention to the surface of the bed (type of bedding, presence of any protruding elements such as brisket boards) than to constraints above ground (stall partitions and neck-rails).

In addition to affecting lying time, stall design also influences standing behaviour. Multiple studies have reported that time spent perching increases if the neck-rail is placed more restrictively (i.e., lower; Fregonesi et al., 2009a), or closer to the rear curb (Bernardi et al., 2009; Fregonesi et al., 2009a), likely because stalls with this configuration are uncomfortable to stand in. Animals also increase time spent perching in scantly bedded stalls (Tucker and Weary, 2004), narrow stalls (Tucker et al., 2004) and stalls with wet bedding (Fregonesi et al., 2007b), which suggests that the behaviour could indicate a reluctance to lie down on uncomfortable surfaces. Conversely, when cows were provided with stalls that have less restrictive or no neck-rail, the animals instead spent more time standing with all four feet in the stall, generally at the expense of time perching (Bernardi et al., 2009; Abade et al., 2015). Similarly, Fregonesi et al. (2009b) reported that when cows were housed on an open pack, they spent more time standing in the lying area, and less time perching and standing idle in the alleyways, compared to when they were housed in a the free-stall system. When animals were given unrestricted access to both conventional stalls and stalls defined by low wooden boards, they preferred to rest in the conventional stalls, while spending more time standing with all four feet in the open stalls (Abade et al., 2015). When simultaneously given access to stalls with different lying surfaces, the animals spent more time lying in stalls with soft bedding (sand, straw), while perching and standing more in stalls with mats and mattresses (Calamari et al., 2009); other work has found that animals compete more for access to stalls with soft surface (Herlin, 1997). Collectively this suggests that dairy cows are well able to evaluate their environment, and choose the most comfortable surfaces for lying and standing. The latter is likely driven by a motivation to avoid hard flooring, as cows prefer soft surfaces when they are standing, walking (Telezhenko et al., 2007) and feeding (Tucker et al., 2006a).

1.4.1.3 Type of flooring

The stall design and the flooring in the home-pen seem to have an interactive effect on the behaviour of dairy cows. The time-budget of the animals changed both for group (Fregonesi et al., 2004) and individually housed (Tucker et al., 2006a) animals when concrete flooring was covered with rubber in the feeding area. The animals spent less time standing (Tucker et al., 2006a) and resting (Fregonesi et al., 2004) in the lying area, and more time standing close to the feed-bunk. Similarly, Boyle et al. (2007) reported that while there was no difference in standing time for animals housed in pens with rubber flooring compared to animals kept on concrete, they spent more time standing outside the stalls. The authors argued that their failure to find any treatment differences in CHDL severity at the end of the study could be explained by the fact that the increased time standing outside the stall counteracted the potentially beneficial effect of softer flooring. However, this explanation should be viewed with caution given the null results of their study. Overall this body of work suggests that changes specifically aimed to improve claw health, such as covering concrete floors with rubber, may fail to decrease lameness if the lying area is poor.

Although strong inferences cannot be made, it is interesting to note that several large cross-sectional studies have found associations between prevalence of lameness and factors that are known to affect lying behaviour, e.g. protruding brisket board (Espejo and Endres, 2007), stall width (Sogstad et al., 2005; Westin et al., 2016), bedding depth (Solano et al., 2015) and hard lying surface (Cook et al., 2004; Dippel et al., 2009). When Rouha-Mülleder et al. (2009) used explorative data mining (regression tree analysis) to investigate the relative importance of different factors associated with lameness (grouped as: housing, management, human-animal interactions, and cow-level factors), factors that generally affect lying time the most (i.e. bedding) had the strongest association with lameness prevalence in small Austrian dairy herds.

1.4.1.4 Climatic conditions

Due to their high metabolic heat production dairy cows are sensitive to heat stress (reviewed by West, 2003). The temperature-humidity index (THI) is one of the most used indicators of heat stress in cattle, and accounts for the combined effect of ambient temperature and relative humidity (RH) on heat load. Based on the relation between climate conditions and decreased milk production, Ravagnolo et al. (2000) suggested that dairy cows become heat stressed at THI 72. This corresponds to a temperature of 25 °C at a relative humidity of 50%, which corresponds to typical daytime conditions during the summer in many areas of Canada (https://www.currentresults.com/Weather/Canada/Cities/temperature-july.php, April 2017). However, behavioural responses to warm conditions occur at even lower THI, and presently THI 68 is suggested as threshold for heat stress in dairy cows (Cook et al., 2007; Allen et al., 2015). Another indicator of heat stress is the black-globe temperature, which in addition to ambient temperature accounts for the effect of solar radiation on the heat load of the animals (Kadzere et al., 2002).

Exposure to excessive heat load elicits multiple physiological responses (reviewed by Kadzere et al., 2002); a reduction in metabolic heat production is achieved by decreased feed intake, while increased respiratory rate and sweating increase heat loss from evaporation. Behavioural changes to alleviate heat stress are also observed during hot weather. Dairy cows are highly motivated to seek shade (Schütz et al., 2008), likely to reduce exposure to solar radiation, and will compete for access if the amount of shade is restricted (Schütz et al., 2010). When providing grazing animals different levels of shade (none vs. shade cloth that blocked 25%, 50% and 99% of solar radiation) animals with access to shade were most likely to seek shelter during times with high ambient solar radiation levels. When the shade cloth blocking 99% of solar radiation was used, the animals spent more time under the shade structure (Tucker et al., 2008). The most commonly performed behaviour during shade use is standing (Kendall et al., 2006; Tucker et al., 2008; Schütz et al., 2010). As such, it is possible that the type of surface under the shade structure could affect claw health. This aspect has not been explored, and could be of interest for farms that allow outdoor access.

Looking at behaviours of housed dairy cows on farms in three different climate zones (Arizona, California and Minnesota), Allen et al. (2015) found that the likelihood of an animal standing up increased with increasing body temperature. This finding provides evidence that prolonged standing is used by the animals to mitigate heat stress, likely by increasing the body surface area available for heat abatement through convection and radiation (Kadzere et al., 2002; Berman, 2003). Cook et al. (2007) repeatedly measured behaviours in a group of animals over the summer season, and found an increase in standing time during periods with warmer weather.

This study also observed a higher proportion of animals being lame towards the end of the warm period, suggesting a link between prolonged standing and lameness. This result aligns with the findings of DeFrain et al. (2013), who evaluated 12-mo foot health records and found a higher prevalence of non-infectious claw lesions during the three months following the hottest period of the year.

When cows were simultaneously given a free choice of four different lying surfaces (soft mat, hard mat, wood shavings and solid manure), De Palo et al. (2006) found that the animals switched their preference from soft mats to organic bedding when THI $\geq$80. Likewise, when comparing groups of animals housed in pens with different types of stall base, the groups kept in pens with organic bedding spent longer time lying when THI >78. These findings could be due to differences in thermal conductivity between the tested bedding materials, suggesting that behavioural responses to heat stress can be influenced by the bedding material used in the stalls. However, as the study was poorly replicated (one pen with eight animals per bedding material), these results should be considered preliminary.

The negative effect of high THI on standing behaviour can be mitigated through heat-abatement strategies such as ventilation, soaking, fogging and a combination thereof (Anderson et al., 2013; Karimi et al., 2015; Porto et al., 2017). It is currently unknown if the use of water for evaporative cooling increases the humidity in the home environment sufficiently to increase horn wear rate, thereby increasing the risk of severe CHDL (Borderas et al., 2004). As heat-abatement strategies are increasingly being implemented on commercial farms, more attention should be directed towards the effects these practices have on claw health and lameness.

In summary dairy cows seem to perceive a soft and dry lying surface as very important, with a weaker preference for less restrictive stall design. Warm climatic condition affects the behaviour of the animals, and might influence what lying surface they prefer. It seems likely that cows use the lying area for multiple purposes, including minimising their exposure to aversive standing surfaces. These results highlight the importance of providing dairy cows the opportunity to both lie and stand on comfortable surfaces (Vokey et al., 2001).

1.4.2 Management factors

1.4.2.1 Milking

Loose-housed dairy cows lack the possibility to rest while waiting to be milked; milking constrains the time-budget of these animals and often requires them to stand for prolonged periods. For animals milked in parlours, it is recommended that the ratio between parlour and pen size allows a group to be milked and returned to the pen within 1 h (DCF-NFACC, 2009), but it is not uncommon that cows spend longer times being milked (0.5 - 6 h/d; Gomez and Cook, 2010; 1.5 - 7.7 h/d; von Keyserlingk et al., 2012; 0.7 - 8.0 h/d; Charlton et al., 2014). Longer times spent away from the home-pen have been associated with higher prevalence of lameness (Espejo and Endres, 2007). Indications for a possible causal link comes from the negative association between time away for milking and daily lying time found in cross-sectional studies (Gomez and Cook, 2010; Charlton et al., 2014). In pens with milking duration exceeding 3.7 h/d, the average lying time was <12 h/d (Charlton et al., 2014).

The amount of time spent standing waiting to be milked also depends on the number of times the cows are milked per day. O’Driscoll et al (2010) found better locomotion in cows kept on pasture and milked once versus twice daily. This aligns with the larger decline in lameness prevalence over time for a group of lame animals milked twice daily, compared to lame animals milked three times per day (Caixeta and Bicalho, 2011). These studies were each performed on just one farm, so it is hard to evaluate the external validity of the findings.

Cook & Nordlund (2009) reported that time spent away for milking is consistent within, but highly variable between animals; some animals repeatedly spend more time outside the pen, and hence have less available time to rest and feed than others. Some studies have reported a weak positive correlation between the dominance order and entry order to the parlour (Soffié et al., 1976; Sauter-Louis et al., 2004). Increasing parity and milk production have inconsistently been correlated with shorter waiting times (Rathore, 1982; Berry and McCarthy, 2012), but for example Gadbury (1975) observed variable effect of parity on entry order between farms. Few studies consider collinearity between different factors (i.e. parity and social status) making it hard to evaluate their relative importance. It is possible that subordinate cows generally need to wait longer to be milked, and hence are more likely to become lame; longitudinal studies are needed to better investigate this relationship.

Lame cows generally spent the longest time waiting to be milked (Hasall et al., 1993; Sauter-Louis et al., 2004; Main et al., 2010), and were slower to return to the home-pen after milking (Juarez et al., 2003). These results suggest that animals that already have impaired locomotion have an increased risk for additional mechanical damage to the claws. This could partially explain the poor treatment outcomes for CHDL (Thomas et al., 2016) and high recurrence rate of sole ulcers (Enevoldsen et al., 1991).

1.4.2.2 Physical constraints

Many farms combine routine management (e.g., veterinary treatment, artificial insemination) with milking (Cooper et al., 2007; Krebs et al., 2011). Although these interventions occur infrequently, they may prolong the duration of forced standing. Experimentally comparing animals that were forced to stand 0, 2, and 4 h, Cooper et al. (2007) found that animals blocked from the lying area for 4 h spent more time lying during the following two days, compared to cows with 0 and 2 h of deprivation. Other studies have reported that animals that were forced to stand also showed more restless behaviour (weight shifting, leg stomping) during the forced standing period (Cooper et al., 2008; Krebs et al., 2011), and had reduced latency to lie down when given access to a lying area (Cooper et al., 2007; Krebs et al., 2011); it is possible that these behaviours are motivated by discomfort in the claws. Together, these results suggest that restricting the duration of forced standing during milking and routine management would be beneficial for claw health.

1.4.2.3 Stocking density

Competition for available resources can alter the time-budgets of dairy cows. The Canadian industry code of practice states that the stocking rate must not exceed 120% for lying stalls, and that enough feed space for animals to meet their nutritional demands must be provided. The code recommends one stall and 0.6 m of linear bunk space/animal (0.76 m/animal if the cows are heavily pregnant; DCF-NFACC, 2009).

Experimental studies generally show a decrease in time spent lying, with a concurrent increase in time standing without feeding, when free-stalls and feed-bunk are overstocked (e.g., Huzzey et al., 2006; Fregonesi et al., 2007a; Hill et al., 2009). The increase in non-nutritive standing is most pronounced directly after fresh feed is provided to the pen (Huzzey et al., 2006), likely because the animals are highly motivated to eat during this period (DeVries and von Keyserlingk, 2005) but not all are able to gain access to feed. Likewise, Winckler et al. (2015) reported that when overstocking the free-stalls, the increase in time spent standing was particularly large at night, which is the most preferred time to rest (Wang et al., 2016).

Compared to a stocking density of 100%, understocking the free-stalls has been found to decrease time spent standing (Telezhenko et al., 2012), but the magnitude of the effect is generally lesser than the effect of overstocking (Fregonesi et al., 2007a; Winckler et al., 2015). However, when housed at 0.82 cows/stall, 63% of the animals spent <12h/d standing, while at stall stocking rates of 1.00 and 1.29 cows/stall, this proportion decreased (Wang et al., 2016). These results indicate that understocking provides more animals the opportunity to rest sufficiently.

The findings discussed so far come from highly controlled studies using small groups of healthy animals, generally exposed to different stocking densities during relatively short time periods (≤2 wk; e.g. DeVries and Keyserlingk, 2006; Krawczel et al., 2012; Telezhenko et al., 2012), and the stocking density has at times been very high (4 cows/feed space; Olofsson, 1999). While these studies have given insights into how overstocking affects the behaviour of dairy cows, it is important that the results also can be applied to the conditions present on commercial dairy farms. Multiple recent cross-sectional studies report a large range of stocking densities for the free-stalls (50 – 200%) and the feed barrier (58 – 228%; von Keyserlingk et al., 2012; Charlton et al., 2014; King et al., 2016; Westin et al., 2016). Charlton et al. (2014) reported that of 111 Canadian farms, no farm with a stall stocking density >100% had an average herd standing time <12 h/d, while 21.6% of the farms with stocking density ≤100% met this target, suggesting that the results from the experimental studies are applicable to the housing conditions on commercial farms.

To evaluate housing conditions common on commercial farms, such as large pens and unstable group composition, Lobeck-Luchterhand et al. (2015) investigated how 80 and 100% stocking density affected the behaviour of dairy cows during the prepartum period. Although dairy cows normally increase the time they spend standing as calving approaches (Huzzey et al., 2005), cows housed at 100% stall stocking density started to increase their standing time one week earlier than animals that were kept at 80% stocking density. In addition, animals in the low-stocked pen never spent as long time standing as the animals in the fully stocked pen.

Despite a positive association between stocking density and CHDL when experimentally overstocking heifers (Leonard et al., 1996), large-scale cross-sectional studies have generally not found an association between stocking density and lameness (e.g. Solano et al., 2015; Westin et al., 2016), possibly because many farms had stall stocking densities lower than 100% in the latter studies. Alternatively, other risk factors (such as the amount of bedding) could have had stronger effects on lameness, making it more difficult to identify any effect of stocking density. Another possible explanation is that the lag time between the mechanical insult and the presence of visible CHDL makes it difficult to identify a potential relationship in cross-sectional studies, as stocking density tends to fluctuate over time. Further work is required to determine if a temporal association exists between stocking density and lameness and CHDL.

1.4.2.4 Regrouping

Regrouping of animals is a common management practice on dairy farms, but regrouping has been found to disrupt the behaviour of dairy cows and increase the time they spend standing

(Hasegawa et al., 1997; Phillips and Rind, 2001; von Keyserlingk et al., 2008). These effects seem to be context dependent, as Talebi et al. (2014) found that regrouping cows to low-stocked pens decreased their standing time. Due to differences in nutrient demand depending on lactation stage, cows are often regrouped multiple times as they transition through the dry period and early lactation; as such, the time period with most frequent regroupings coincides with the periparturient increase in laxity of the suspensory apparatus.

In summary, management choices affect the time-budgets of dairy cows. Stocking density >100% in the lying and feeding areas generally result in longer standing time, as does regrouping of the animals.

1.4.3 Animal factors

1.4.3.1 Stage of lactation

As cows approach calving they change their behaviour. These behavioural changes include prolonged standing, increased walking, restlessness, and more frequent transitions between lying and standing (Jensen, 2012). Reports of how long before calving standing time starts to increase are variable (1 vs. 16 d; Huzzey et al., 2005; Calderon and Cook, 2011). The disparate results were not caused by differences in stocking density between the studies (95 – 100% stall stocking density and 0.6 m linear feed space/cow in both studies), but it is possible that differences in what bedding material was used (deep-bedded sand vs. mattresses) influenced the behaviour of the animals. During the last 24 h before calving the standing duration is notably longer, with an increase in the number of transitions between standing and lying starting approximately 6 h before calving (Miedema et al., 2011; Jensen, 2012). Campler et al. (2014)

reported that dairy cows showed a partial preference for calving on sand compared to rubber mats even though deep-bedded straw covered both stall bases, possibly because the sand provided better traction when the cows transitioned between standing and lying. These findings highlight the importance of pen designs that promote lying, and that provide soft standing surfaces with good traction in periparturient housing.

Multiple studies have reported that time spent standing decreases from early to late lactation (e.g. Deming et al., 2013; Ito et al., 2014). In addition, Løvendahl and Munksgaard (2016) measured the time-budgets of primiparous cows in early and late lactation, and found that high-producing animals spent more time feeding and less time lying during both periods. Given that dairy cows are highly motivated to maintain lying time (Jensen et al., 2005), this finding suggests a high level of competing time demands for high-yielding cows. Österman and Redbo (2001) reported that cows milked twice daily spent more time standing during the 4 h directly before morning milking compared to animals milked three times daily, and suggested that increased udder fill may cause discomfort when the cows are lying.

1.4.3.2 Disease

During the transition period dairy cows undergo physiological and metabolic changes due to parturition and onset of lactation (Grummer, 1995). It is possible that the combined effect of metabolic and management stressors explain the disproportionally high incidences of disease (LeBlanc et al., 2006) seen during the first month of lactation. Many diseases have been found to influence standing behaviour; prolonged standing has been associated with mastitis (Siivonen et al., 2011; Medrano-Galarza et al., 2012), subclinical hypocalcaemia (Jawor et al., 2012), and clinical ketosis (Itle et al., 2015). It is uncontroversial that lameness negatively affect health and production (reviewed by Huxley, 2013), but the findings of longer standing time in sick animals raise the interesting notion that this relation could be bidirectional.

Overall the reviewed studies indicate that numerous cow-level factors influence the standing behaviour of dairy cows. The way dairy cows interact with their environment is not static, but rather shows general and predictable fluctuations over the lactation cycle. However, the large variation in daily standing time between animals (4.5 - 19.8 h/d; Ito et al., 2009) illustrates that the exposure to environmental risks, such as hard flooring, is not evenly distributed among the cows.

1.5 The social environment

In stable groups of dairy cows the frequency of agonistic interactions (threats, avoidance, physical aggression) is generally low, but during periods with increased social stress, such as regrouping or overstocking, the number of aggressive interactions increases (e.g. DeVries et al., 2004; Keyserlingk et al., 2008). Galindo and Broom (2000) suggested that keeping dairy cattle in intensive housing systems may affect their social behaviour, resulting in higher risk of lameness in subordinate animals. The aims of this section are to contrast the social structure of housed dairy cows with that of free-ranging cattle, and to critically evaluate what is currently known about how social status influences standing behaviour. The section ends with a description of what is published on the effects of the social environment on the risk of CHDL and lameness.

1.5.1 The social structure of cattle

Cattle are highly gregarious animals, and respond behaviourally when isolated from their group (e.g. by escape attempts; Purcell and Arave, 1991; and vocalizing; Boissy and Le Neindre, 1997). Groups of cattle form social hierarchies (Reinhardt and Reinhardt, 1981; Wierenga, 1990), which are regulated through agonistic interactions between pairs (dyads) of animals (Beilharz and Zeeb, 1982; Val-Laillet et al., 2008a). Within each dyad, an animal is considered dominant by the extent it can influence the behaviour of the other animal (Beilharz and Zeeb, 1982), or is favoured by the outcome of agonistic interactions (Reinhardt et al., 1986; Drews, 1993).

Many studies have found that older animals (e.g. Schein and Fohrman, 1955; Šárová et al., 2013), and animals with higher body weight (Reinhardt and Reinhardt, 1975) are more likely to be dominant. However, these factors are often confounded with seniority in the herd. Studies introducing new animals to an existing group generally report that the newcomers were able to dominate a lower proportion of animals than what would be expected from their age and size (dairy cattle; Schein and Fohrman, 1955; beef cattle; Mench et al., 1990). Counterintuitively, the amount of aggression an animal direct towards other cows is poorly correlated with the social rank of the animal (Reinhardt and Reinhardt, 1975; Reinhardt et al., 1986). There is some evidence that territory ownership gives advantages; Hasegawa et al. (1997) found that residential cows increased the number of animals they were able to dominate after regrouping, while the opposite was found for cows introduced to the group.

Once a dyadic dominance relationship is formed it generally remains stable (dairy cattle; Reinhardt and Reinhardt, 1975; Beilharz and Zeeb, 1982; Wierenga, 1990; beef cattle; Wagnon et al., 1966; Reinhardt et al., 1986; Šárová et al., 2013; feral cattle; Hall, 1986). This is believed to benefit both dominant and subordinate animals, as being able to predict the outcome of encounters reduces the risk of getting injured in fights (Reinhardt et al., 1986; Wierenga, 1990). All dyadic dominance relationships combined results in the observed rank order at the level of the group (Beilharz and Zeeb, 1982); an animal that dominates a higher proportion of the group members will have a higher social rank. Perfectly transitive relationships, i.e. if A dominates B, and B dominates C, then A dominates C (A>B>C<A), result in linear social hierarchies.

1.5.1.1 Free-ranging cattle

Free-ranging cattle with little human contact divide into spatially separated, stable groups (herds) that show strong social affinity within the group (Lazo, 1994). Multiple observational studies have reported that the most closely and consistently affiliated animals within the herd were adult cows and their offspring (semi-wild Zebu cattle; Reinhardt and Reinhardt, 1981; Doñana feral cattle; Lazo, 1994; semi-wild Highland cattle; Reinhardt et al., 1986; domesticated Salers cattle; Veissier et al., 1990). Both in *Bos indicus* (Reinhardt and Reinhardt, 1981) and *Bos taurus* (Veissier et al., 1990), older offspring showed greater preference for their younger siblings compared to unrelated calves. While female offspring generally remained in the herd they were born in (Lazo, 1994), bulls typically left the maternal group and formed small, less stable, male groups when they became mature (Hall, 1986). The male groups only showed seasonal affiliation with the matriarchal herds (Lazo, 1994).

Studies evaluating the dominance structure of free-ranging cattle consistently report imperfect, but strongly linear social hierarchies (feral Chillingham cattle; Hall, 1986; semi-wild Highland cattle; Clutton-Brock et al., 1976; Reinhardt et al., 1986; beef cattle; Wagnon et al., 1966; Šárová et al., 2013; dairy cattle; Schein and Fohrman, 1955). However, these results should be interpreted with caution, as only two studies had accounted for unknown dyadic relationships (Hall, 1986; Šárová et al., 2013), and missing data increase the risk of erroneously identifying dominance structures as linear (Appleby, 1983).

Reversals of dyadic dominance relationships were reported to occur infrequently, with 80 – 99% of known relationships between adult females remaining stable between years (Wagnon et al., 1966; Hall, 1986; Šárová et al., 2013). Wagnon et al. (1966) found a rank-correlation coefficient of 0.95 between rank orders measured on subsequent years, indicating that reversals of dyadic dominance relationships mainly occur in pairs that are close in rank. Bidirectional relationships, when both animals win at least one agonistic encounter, were uncommon (1.5 – 3.2% of possible female pairs; Schein and Fohrman, 1955; Hall, 1986), as were pair-wise relationships in which the animal with lower social rank was dominant (1.0 – 2.1% of possible pairs; Schein and Fohrman, 1955; Clutton-Brock et al., 1976; Reinhardt et al., 1986). Results from studies that in a standardised way reported the rate of agonistic behaviours suggest that aggression occurs infrequently in free-ranging cattle (0.1 – 0.7 agonistic interactions/cow-hour ; Clutton-Brock et al., 1976; Hall, 1989; Kabuga et al., 1991); with most agonistic behaviours directed towards animals close in rank (Reinhardt et al., 1986; Kabuga, 1992).

1.5.1.2 Loose-housed dairy cattle

For loose-housed dairy cattle in intensive production systems the social environment is mainly determined by management decisions. This generally results in the elimination of close dam-calf bonds, and recurrent regroupings frequently disrupt the social stability of the group. Under these circumstances, both experimental (Broom and Leaver, 1978; Patison et al., 2010) and observational (Gutmann et al., 2015) studies indicate that familiarity, rather than kinship, is important for cohesion between animals. Despite frequent disturbances in the social environment, dyadic dominance relationships were relatively stable also in loose-housed dairy cattle, with 75 – 85% of the relationships between adult females remaining stable over 2 y (Reinhardt and Reinhardt, 1975; Wierenga, 1990). The social hierarchy was less transitive and more complex compared to free-ranging cattle, and had a high proportion of bidirectional relationships (41 – 66% of possible dyads; Wierenga, 1990; Val-Laillet et al., 2008, 2009), and circular triads (A>B>C>A; 45 – 58% of possible triads; Val-Laillet et al., 2008, 2009). An interesting finding of Val-Laillet et al. (2008b) was that the competitive success at different types of resources (i.e., the feed-bunk, the free-stalls and a mechanical brush) showed low correlation within individual animals, suggesting that a cow may be more or less willing to compete for a resource depending on how valuable it is perceived.

It is likely that the more complex hierarchy observed in loose-housed dairy cattle is a function of how they are managed. Frequent regroupings increase the chance that external factors (e.g. advantage of home territory) become influential during the formation of dyadic dominance relationships (Kokko, 2013). Regroupings may bring together animals whose pair-wise relationships have developed independently during one of several previous mixings. This could result in circular triads if A previously dominated B and B previously dominated C, but after regrouping A becomes subordinate to C while maintaining dominance over B (Schein and Fohrman, 1955; Wierenga, 1990).

Even if bidirectional relationships were frequently observed, Wierenga (1990) reported that only 3.9% of all displacements were performed by the subordinate animals. This result suggests that contradictory displacements (i.e. the low-ranked cow displaces an animal with higher social rank) may not primarily occur because of social rank competition. As feeding space decreased, the frequency of contradictory displacements increased (0.3 displacements/cow-day at 100% vs. 0.5 displacements/cow-day at ≥125% stocking density; Wierenga, 1990), as did the proportion of dyads for which contradictory displacements were observed (65.5% vs. 86.3% of possible dyads at 100% vs. 200% stocking density; Val-Laillet et al., 2009). In addition, Wierenga (1990) found that animals that were displaced more often from the feed-bunk also performed more contradictory displacements. Taken together these results suggest that sufficient motivation to gain access to a limited resource can override the restraining effect of lower social rank, and elicit displacements of more dominant animals (Wierenga, 1990; Val-Laillet et al., 2008a).

The differences in dominance structure between free-ranging and loose-housed cattle implies that animals kept in intensive housing systems live under higher social tension; this is further substantiated by the lower rate of agonistic interactions that was reported by Miller and Wood-Gush (1991) when loose-housed dairy cattle were kept on pasture, compare to when they were housed indoors (pasture: 1.1 interactions/cow-hour vs. indoors: 9.5 interactions/cow-hour).

1.5.2 Behavioural responses are influenced by social status

Multiple experimental studies report that the negative effects of regrouping and overstocking are particularly severe for socially subordinate animals (e.g. Hasegawa et al., 1997; Devries and Keyserlingk, 2006; Winkler and Margerison, 2012). Heifers and primiparous cow generally have low social rank (Krohn and Konggaard, 1979; Reinhardt et al., 1986), and are less successful when they need to compete with older animals in mixed groups (Phillips and Rind, 2001; González et al., 2003). Low-ranked cows have been found to adjust their time-budget to a higher extent than dominant animals during periods with high social stress (e.g. DeVries et al., 2004; Huzzey et al., 2006; Winckler et al., 2015).

When overstocking the feed-space, Olofsson (1999) found that subordinate animals spent more time lying and standing without feeding during peak feeding time. Low-ranked animals were more often displaced from the feeding stations, and might have chosen to avoid this area during the time when most agonistic interactions took place. When using a y-maze choice test Rioja-Lang et al. (2012) found that animals with low competitive success at the feed-bunk were willing to give up high quality food in the presence of a dominant animal, when the linear feeding space was <0.6 m. Fregonesi et al. (2007) reported that when cows were kept at 150% stall stocking density, some animals chose to lie down instead of feeding after returning from milking, even though adequate feeding space was provided for all animals. Although social status was not assessed in this study, another study found that animals with low competitive success at the stalls were more likely to increase the time they spent lying during daytime (Winckler et al., 2015), which is a less preferred time for rest in dairy cows (Wang et al., 2016). Wierenga and Hopster (1990) reported that subordinate animals could maintain normal lying times up to 133% stocking density, by increasing the time they spent resting during less preferred periods of the day. At higher stocking densities the standing time increased for low-ranked animals, while dominant animals were largely unaffected.

1.5.3 Social status and CHDL

Galindo and Broom (2000) reported that subordinate animals were more likely to become lame during the indoor housing period, possibly because of their lower competitive success and their longer time spent perching and standing idle in the alleyways. In the following sections I will discuss common management practices that can pose social challenges for the cows, from the perspective of CHDL and lameness risk.

1.5.3.1 Automatic milking systems (AMS)

For automatic milking systems (robotic milking) to function satisfactorily, the cows must voluntarily walk to the milking robot. For this reason, the home-pen is usually constructed to ensure frequent visits to the milking unit. The majority of robotic farms operate either by encouraging the animals to freely seek out an milking unit placed in an open area of the pen (free cow traffic), or by installing selection gates that direct the animals either to the waiting area in front of the milking unit or back to the resting and feeding areas, depending on if it needs to be milked or not (guided cow traffic).

Witaifi et al. (2018) reported that overstocking lead to increased number of displacements at the free-stalls and feed-bunk also in AMS, and multiple studies have found that the social status affects the time-budget of dairy cattle in this type of housing system (Ketelaar-de Lauwere et al., 1996; Melin et al., 2006; Lexer et al., 2009). While dominant animals more often could access the milking unit directly after they had entered the waiting area (Ketelaar-de Lauwere et al., 1996), subordinate animals spent more time standing in front of the robot before they were milked (Ketelaar-de Lauwere et al., 1996; Melin et al., 2006; Lexer et al., 2009). Increased time waiting to be milked has been observed for subordinate animals even when the milking unit was stocked at 50% of recommended capacity (Lexer et al., 2009).

Ketelaar-de Lauwere et al. (1996) reported that subordinate animals had a higher proportion of milkings during night-time. Primiparous cows housed separately from older animals were milked more frequently, and had a lower proportion of involuntary milkings (handlers fetching animals for milking), than first parity animals mixed with multiparous cows (Bach et al., 2006). These results indicate that low-ranked animals may avoid the milking unit when dominant animals are present. Simulation studies, based on farm data, have been used to explore how cow traffic changes if milking is backed up (e.g. due to maintenance work on the milking unit); the results indicate that the waiting time would disproportionate increase for subordinate animals (Halachmi, 2009).

So far, little research has evaluated the relationship between the social environment and lameness when the cows are milked in AMS. In a cross-sectional study of robotic farms, King et al. (2016) reported that the prevalence of severe lameness was positively correlated with stocking density, suggesting that more work exploring this topic is warranted.

1.5.3.2 Transition into first lactation

Heifers often enter the main herd either in late gestation or soon after first calving, and may experience a new housing system and milking-related handling for the first time during the same period. There is a large variation in how dairy farms transition their heifers into the milking herd, suggesting that many farmers find it challenging to manage this period in a good way (Espadamala et al., 2016). In an epidemiological study Barker et al. (2007) found that farms that housed prepartum heifers with the milking herd instead of with the dry cows had higher herd locomotion scores. Unfortunately, the locomotion scores were not recorded at cow-level, so it is not possible to know if the higher locomotion scores were caused by impaired gait in the heifers specifically.

With the aim of finding regrouping strategies that minimise social stress, various ways of introducing heifers to the milking herd have been evaluated in experimental studies. Pair-wise introduction (Neisen et al., 2009), introduction during low-activity periods of the day (Boyle et al., 2012), and previous co-housing with multiparous cows (Boyle et al., 2013) reduced the frequency of agonistic behaviours experienced after mixing. However, the studies that have measured standing behaviour in heifers all report prolonged standing after mixing with multiparous cows (on average >7 h the first 8 h; O'Connell et al., 2008; >20 h the first 24 h; Boyle et al., 2012, 2013; approx. 30 h the first 33 h; Wagner et al., 2012). Similarly, Krohn and Konggaard (1979) reported longer standing times after regrouping for pre-calving heifers mixed with multiparous cows, compared to heifers mixed with primiparous animals.

Most of the studies only measured behavioural responses to regrouping, so information regarding how these responses are related to lameness and CHDL in heifers is limited. O'Connell et al. (2008) did assess CHDL, and found that newly calved heifers introduced in pairs to the milking herd showed most activity after regrouping, and subsequently developed more severe claw horn lesions.

As prolonged standing increases the mechanical load on the claw, it is possible that separating the introduction of heifers to the main herd from the time period with increased laxity in the suspensory apparatus could have beneficial effects on their claw health. Alternatively, the mechanical load during this sensitive period could potentially be reduced by providing the animals with a soft standing surface. Previous work has reported that newly calved heifers housed on deep-bedded straw (Webster, 2002), or in pens with slatted rubber flooring (Bergsten et al., 2015), after calving developed less severe claw lesions during their first lactation. However, both of these studies were poorly replicated, so the results should be considered preliminary.

1.6 Gaps in the literature

While abundant experimental work shows that heifers and subordinate animals change their behaviour to a larger degree in competitive environments, less research has evaluated if this will translate to an increased risk of lameness and CHDL for these animals. The prolonged periparturient standing time that has been reported for cows that developed severe sole lesions later in lactation (Chapinal et al., 2009b; Proudfoot et al., 2010) suggests that the transition period is of particular interest. However, since these two studies were conducted at the same research facility, it should be verified that the relationship between prolonged standing during the transition period and development of sole lesions is present also during the less controlled conditions on commercial farms. Although multiple studies have evaluated different strategies for introducing heifers into the milking herd, no research has evaluated if a low-stress social environment would be beneficial for claw health. In conclusion, more attention should be directed to how the social environment affects the claw health and welfare of dairy cows.

1.7 Thesis objectives

Based on the identified gaps in the literature, the overall aims of this thesis were to: 1) evaluate the relationship between prolonged standing and long standing bouts around calving and sole lesion development later in lactation on commercial farms, and 2) assess the effects of the social environment after regrouping on the behaviour and claw health of newly calved heifers. As there is currently little research that can guide the interpretation of longitudinal locomotion data, an additional objective was to determine what effects the lameness definition and the frequency of locomotion assessment have on measures of lameness incidence.

I hypothesised that more animals would be categorised as lame when using a less stringent lameness definition, and when assessment occurred more frequently, resulting in higher estimates of lameness incidence (Chapter 2). Furthermore, I hypothesised that dairy cows with prolonged standing time, or long standing bouts, during the transition period would be at higher risk of developing sole lesions (Chapter 3), and that new-calved heifers regrouped to a low-stocked pen with familiar peers of the same age would spend less time standing and perching after regrouping, than would heifers mixed with multiparous cows after calving (Chapter 4).

Chapter 2: Effects of case definition and assessment frequency on lameness incidence estimates

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2.1 Introduction

Lameness is one of the greatest welfare challenges for dairy cows, and many cases are associated with claw lesions (Archer et al., 2010). Despite decades of research lameness remains a common disorder (Bicalho and Oikonomou, 2013), perhaps in part due to the difficulty in correctly identifying lame animals. Intra- and inter-observer reliability estimates often indicate low to moderate consistency when assessing locomotion (reviewed by Schlageter-Tello et al., 2014b), and it is particularly difficult to differentiate between imperfect gait and mild lameness (Schlageter-Tello et al., 2014a). This inconsistency introduces uncertainty into measures of prevalence and incidence.

Point prevalence estimates (i.e. the proportion of animals that are lame at a specific time point) from cross-sectional studies have frequently been used to evaluate associations between potential risk factors and lameness (e.g. Faull et al., 1996; Barberg et al., 2007; Solano et al., 2015). Incorrectly classified animals (both sound and lame) increase the risk that true correlations are overlooked, or that spurious relationships are identified.

Cross-sectional studies can only conclude bidirectional associations (Dahoo et al., 2009), so in recent years more effort has been made to evaluate the temporal associations between risk exposure and lameness using longitudinal studies (e.g. Green et al., 2014; Randall et al., 2016). However, in the light of poor observer reliability, it is unclear how much weight should be put on single gait scores. To address this issue, some longitudinal studies have used a more stringent definition of lameness (e.g. two consecutive occasions scored as mildly lame, or one occasion scored as severely lame; Randall et al., 2015, 2017), but to our knowledge the effect of lameness definition on diagnosis of new lameness cases has not been evaluated.

Additionally, there are currently no recommendations for how often gait should be assessed to identify new cases of lameness in longitudinal research. Assessment interval varies considerably between lameness studies (i.e. monthly; Frankena et al., 2009; weekly; Randall et al., 2017), making it hard to compare their results.

Using data from dry cows on commercial farms, the objectives of this paper were to evaluate how lameness definition and assessment frequency affect identification and classification (mild or severe lameness) of new lameness cases. We hypothesised that more animals would be categorised as lame when using a less stringent lameness definition, and when assessment frequency increased, resulting in a higher estimate of incidence. Claw lesions have been imperfectly associated with lameness (Flower and Weary, 2006; Chapinal et al., 2009b) and lameness is commonly used to identify cows in need of treatment (Whay, 2002). As such, we also compared the evaluated lameness definitions against trim records from trimmings occurring 90 d or less before calving.

2.2 Material and methods

2.2.1 Farm selection and description

This study used data from a study evaluating how lameness during the dry period affect the risk of transition period diseases (Daros et al., 2018), approved by the Animal Care Committee at the University of British Columbia (protocol A15-0084). A convenience sample of six commercial dairy farms in the lower Fraser Valley region (BC, Canada) were visited weekly between May and December 2017. Farms were recruited by partnering with a local claw trimming company (AR-PE Hoof Trimming Ltd., Abbotsford, BC, Canada). Enrolled farms housed adult cows predominantly in free-stall pens (3 farms used deep-bedded open packs for a short period at the end of the dry period), kept dry animals separate from lactating, and had 153 – 543 lactating cows at the beginning of the study. Five farms only kept one breed (Holstein), while one farm had a mix (Holstein/Jersey/Ayrshire).

2.2.2 Enrolled animals

To evaluate how case definition and assessment frequency affect measures of lameness incidence, only data from the 271 parous pregnant animals that were sound at first assessment and kept on-site during the full dry period were used in the analyses. The study population was therefore not a representative sample of loose-housed dairy cows, and our results should be interpreted in the context of the study objectives. Each cow was enrolled 9 wk before the estimated calving date. Due to early calving, 33 cows (12.6%±2.7% of animals/farm; mean±SD) were first assessed 8 wk before actual calving date. As later enrolment would have provided insufficient locomotion data, we excluded nine animals that had their first assessment <8 wk prepartum, leaving 262 cows in the data set. A median of 49 animals (range: 9 – 73) were followed/farm.

2.2.3 Locomotion assessment

Before data collection started two observers with previous experience in gait scoring (GS) were trained to provide consistent ratings using an ordinal scale from 1 to 5 (GS1 = smooth and fluid movement, GS2 = imperfect locomotion, GS3 = compromised locomotion, GS4 = obviously diminished ability to move freely, and GS5 = severely restricted ability to move; Flower and Weary, 2006). Initial training in identification of abnormal gait characteristics (head-bob, back arch, shortened track-up, asymmetrical weight bearing, and limp), and overall locomotion scoring, was done using video recordings of cows walking in a straight line on dry concrete. This was followed by training in live observation as used in this study at the UBC Dairy Education and Research Centre. The inter-observer reliability was evaluated after each step of the training protocol (see Table 2.1 and Table 2.2 on page 45). Evaluating many animals might exhaust observers, and negatively impact the assessment accuracy (Schlageter-Tello et al., 2015), thus we controlled for the number of animals used for the live reliability assessment.

Inter-observer reliability for ordinal gait assessment was evaluated using weighted kappa (Cohen, 1968) with linear and quadratic weighting (Vanbelle, 2016). Using the framework of Landis and Koch (1977), the kappa values indicated substantial to almost perfect agreement (linear weighting: $\boldsymbol{K}_w$ = 0.76, 95% CI 0.53 - 0.99; quadratic weighting: $\boldsymbol{K}_w$ = 0.84, 95% CI 0.69 - 1.00) for video assessments, and moderate agreement (linear weighting: $\boldsymbol{K}_w$ = 0.52, 95% CI 0.24 - 0.80; quadratic weighting: $\boldsymbol{K}_w$ = 0.57, 95% CI 0.28 - 0.87) was achieved when evaluating inter-observer reliability for live scoring.

Table 2.1 Inter-observer agreement for gait scoring from video

		Observer 1					
		1	2	3	4	5	Total
	1	0	0	0	0	0	0
	2	1	9	1	0	0	11
Observer 2	3	0	1	4	0	0	5
	4	0	0	1	3	0	4
	5	0	0	0	0	0	0
	Total	1	10	6	3	0	20

Table 2.2 Inter-observer agreement for gait scoring at live training

		Observer 1					
		1	2	3	4	5	Total
	1	0	0	0	0	0	0
	2	0	20	3	0	0	23
Observer 2	3	0	7	9	0	0	16
	4	0	0	0	1	0	1
	5	0	0	0	0	0	0
	Total	0	27	12	1	0	40

To evaluate the effects of prevalence and observer bias on the reliability measures locomotion data was dichotomized as sound (<GS3) and clinically lame (GS≥3; see Table 2.3 and Table 2.4 on page 46), and the prevalence index (**PI**) and bias index (**BI**) were calculated for video and live training as described by Byrt et al. (1993). The indexes can take values between -1 and 1, with a PI of 0 indicating that an equal number of animals were classified as sound and lame, and a BI of 0 indicating that there was no systematic bias between the observers. For video assessment, PI was 0.1 and BI was 0.0, while for live evaluation, PI was 0.3 and BI was -0.1.

Table 2.3 Inter-observer agreement for lameness identification from video

		Observer 1		
		Sound	Lame	Total
Observer 2	Sound	10	1	11
	Lame	1	8	9
	Total	11	9	20

Table 2.4 Inter-observer agreement for lameness identification at live training

		Observer 1		
		Sound	Lame	Total
Observer 2	Sound	20	3	23
	Lame	7	10	17
	Total	27	13	40

Reliability for live scoring was reassessed after the data collection was completed (linear weighting: $\boldsymbol{K}_w$ = 0.49, 95% CI 0.19 - 0.80; quadratic weighting: $\boldsymbol{K}_w$ = 0.55, 95% CI 0.23 - 0.87; Table 2.5), with PI and BI values of 0.2 and 0, respectively (see Table 2.6 on page 47). The similar results indicate that the observers maintained reliability, and did not develop systematic bias over the study period.

Table 2.5 Inter-observer agreement for gait scoring after study completion

		Observer 1					
		1	2	3	4	5	Total
Observer 2	1	0	0	0	0	0	0
	2	0	17	5	0	0	22
	3	0	5	9	0	0	14
	4	0	0	0	1	0	1
	5	0	0	0	0	0	0
	Total	0	22	14	1	0	37

Table 2.6 Inter-observer agreement for lameness identification after study completion

		Observer 1		
		Sound	Lame	Total
Observer 2	Sound	17	5	22
	Lame	5	10	15
	Total	22	15	37

During the study locomotion was scored weekly during pen walks when unrestricted animals walked on concrete while being observed directly and obliquely from behind by one of the observers. Farmers were informed about animals with ≥GS3, and treatment was initiated at the discretion of the farm manager.

2.2.4 Claw lesion recording

Farms were visited every 2 – 10 wk by the same trimming company through which the farms were recruited. The cows were trimmed by one of three professional trimmers who also identified claw lesion in accordance to Alberta Dairy Hoof Health Project's Lesion Severity Scoring Guide (*dairyhoofhealth.info/Lesion-Severity-Guide-v0.7.pdf*), using the Hoof Supervisor System™ software (KS Dairy Consulting, Inc., Dresser, WI, USA). On five of the enrolled farms cows were routinely trimmed before dry off, while on the sixth farm animals were selected for trimming based on toe length. Records for trimmings occurring during 2017 were downloaded after the on-farm data collection was completed, and lesion records from trimming occurring ≤90 d before calving, including during the dry period, were extracted (data were available for 117 of the 262 enrolled cows). Data were summarised per animal as either having, or not having, non-infectious (sole haemorrhage n = 1, sole ulcer n = 3, white line lesion n = 4, toe ulcer n = 2; one animal had >1 type of claw horn lesion) and infectious claw lesions (digital dermatitis n = 22, interdigital dermatitis n = 6; two animals had both types of infectious lesions).

2.2.5 Data handling and statistical analyses

Lameness definitions

To evaluate the effects of the case definition on identification of new lameness cases, clinical lameness was defined either as 1) scored GS3 or higher on at least one occasion (**LAME1**); 2) scored GS3 on at least two consecutive occasions or scored GS4 or higher at least once (**LAME2**); or 3) scored GS3 on at least three consecutive occasions or scored GS4 or higher at least once (**LAME3**). Using one score ≥GS4 was justified by the relative ease of identifying animals with severely restricted ability to move (Schlageter-Tello et al., 2014a), making it unlikely that sound animals would be scored GS4 or higher. LAME3 was treated as the gold standard for clinical lameness, and ≥GS4 were used to define severe lameness (Flower and Weary, 2006).

Assessment frequency

When evaluating the effects of assessment frequency every (**ASSM1**), every other (**ASSM2**), or every third (**ASSM3**) assessment were retained for each animal, with the first assessment being the same in all three data sets. For cows that were gait scored for the first time 9 wk before calving (87% of enrolled animals) four observations remained in ASSM3, while animals first observed 8 wk prepartum (13%) were scored three times. After creating the three data sets, the case definition found to have the best test characteristics given the conditions of our study was used to categorize cows as sound or lame; ASSM1 was used as the gold standard.

2.2.5.1 Statistical analyses

All data analyses were performed in R version 3.4.4 (R Core Team, 2018; RStudio Team, 2016; Wickham et al., 2017; Wickham and Henry, 2018). Graphing was performed using the ggplot2 package (Wickham, 2009). Mixed logistic regression models with farm as random intercept were fitted with maximum likelihood using the lme4 package (Bates et al., 2015) to estimate sensitivity (proportion of true positives that were correctly identified by the test), specificity (proportion of true negatives correctly identified by the test), positive predictive value (**PPV**; probability of correct positive test), and negative predictive value (**NPV**; probability of correct negative test) as described by Coughlin et al. (1992). The uncertainty of the estimates was evaluated with exact binomial 95% confidence intervals (Dahoo et al., 2009). Percent agreement was calculated from contingency tables. Reliability in the classification of lameness severity (sound, mildly lame, severely lame) was evaluated with weighted kappa (linear and quadratic weighting; Vanbelle, 2016), using the irr (Gamer et al., 2012) and rel (Lo Martire, 2017) packages. The kappa statistic assumes that the data are independent, which has generally been interpreted as observers having no knowledge of previous assessments (Sim and Wright, 2005), and that repeated assessments of the same subject must be accounted for (Haley and Osberg, 1989). To evaluate if dependencies within the data affected the reliability estimates, kappa values were first obtained for all animals, and then separately for the three largest herds (n = 50 – 73).

As the three lameness criteria were nested, it was not possible to estimate their relative test attributes with logistic regression due to complete data separation; as such, only percent agreement and weighted kappa statistic were used when evaluating the correspondence between the definitions. However, logistic regression models were used when comparing the different lameness criteria against recorded claw lesions. Logistic regression, contingency tables, and weighted kappa were used when evaluating the effects of assessment frequency on identification of animals that became lame.

As lameness incidence was calculated from a closed population, incidence proportion was used as the outcome (number of cows becoming lame/total number of cows; Dahoo et al., 2009). Differences in farm incidence depending on lameness definition and assessment frequency were tested using paired t-tests. The low number of animals for one of the farms (n = 9) increased the uncertainty in the incidence estimate for this farm; all analyses were therefore run with and without animals from this farm. This caused no substantial differences in the results, so analyses using the full data set are presented here.

2.3 Results and discussion

2.3.1 Locomotion assessment

The high weighted kappa values after video training suggest that the observers were consistent in the way they differentiated between gait scores. The lower values for the linear-weighted kappa compared to the quadratic-weighted kappa indicate that disagreements generally consisted of one GS (Vanbelle, 2016). The consistently low BI values suggest that the lower kappa values obtained for live scoring were not due to systematic differences in how the observers scored locomotion, but rather that it was difficult to differentiate between GS2 and GS3 (see Table 2.1 and Table 2.2 on page 45; Vach, 2005). This finding aligns with Schlageter-Tello et al. (2015), who reported the lowest intra-observer percent agreement for GS2 and GS3 scores. Taken together, these results emphasise that the interpretation of longitudinal locomotion data should account for observer inconsistency.

On ten occasions, locomotion assessment could not be performed (0.4% of all occasions); on eight occasions cows calved during the visit, and on two occasions animals were not found in their home-pen; no animal lacked more than one locomotion score. The percentage of locomotion scores of 1, 2, 3, 4, and 5 were 0%, 69.5%, 26.5%, 4%, and 0%; with 18%, 60%, and 22% of the animals having a maximum score of 2, 3, and 4, respectively. The reason that no animal was scored as having perfect gait may have been that the home-pens did not provide optimal conditions for walking freely (other animals present, manure contaminated concrete; Telezhenko et al., 2017). It were common for locomotion scores to change from one visit to the next; approximately a third of the scores changed between consecutive assessments. In agreement with Reader et al. (2011), scores were much more likely to change by one unit (e.g. from GS2 to GS3, or GS3 to GS4; 94% of all changes) than by two or more units (e.g. from GS2 to GS4). Inconsistency within and between observers could explain some of these changes (Figure 2.1a and Figure 2.1c on page 52), as well as animals transitioning between being sound and becoming lame and vice-versa (Figure 2.1d and Figure 2.1e).

2.3.2 Effect of lameness definition

The number of animals categorised as becoming lame depended on what definition that was used [LAME1: n = 215 (82% of enrolled animals); LAME2: n = 131 (50%); LAME3: n = 93 (35%)]. These differences were due to animals that were considered sound when using the LAME3 criteria (n = 169), while being classified as mildly lame when LAME1 (122 of 169 animals), or LAME2 (38 of 169 animals) definitions were used. The number of severely lame animals (n = 57) did not differ, as the criterion for severe lameness was the same in all definitions. The high proportion of animals categorised as lame when using LAME1 suggests that the lameness incidence estimates were inflated when using this criterion, especially considering that 44 of the 158 mildly lame (28%) LAME1 animals were scored GS3 on only one occasion.

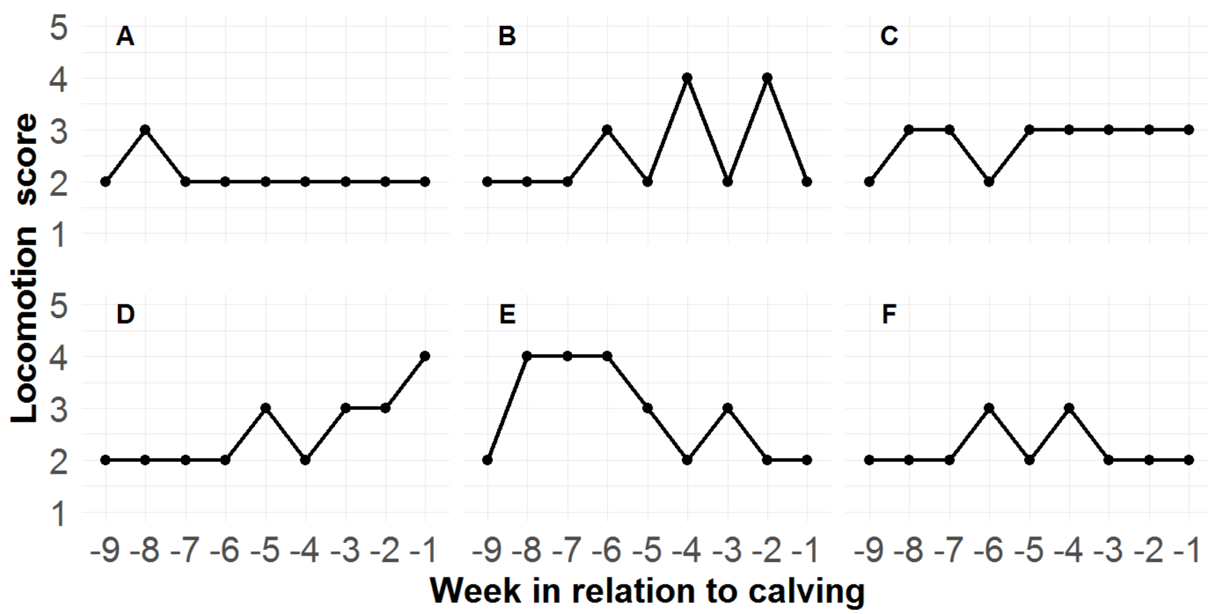

Figure 2.1 Examples of gait patterns
Gait trajectories for six different animals, illustrating changes in weekly gait scores over time. When one occasion ≥GS3 was used to define lameness cow A was considered a new case of lameness, contributing to farm incidence. With decreasing assessment frequency, it became harder to identify all lame animals; cow B was categorised as sound when evaluated every 2 wk despite multiple scores of GS4, and cow C when assessed every 3 wk (mild lameness here defined as ≥2 consecutive GS3). The presence of isolated GS2 scores in the beginning (D), and end (E) of severe lameness events suggests that it is difficult to distinguish between GS2 and GS3 in marginally lame animals, which raises the question of how to interpret the trajectory for cow F.

The high percent agreement (see Table 2.7 on page 53) between LAME2 and LAME3 suggests that animals that were scored GS3 or greater on two consecutive occasions were likely to be scored ≥GS3 also a third time. This was also reflected by weighted kappa values indicating almost perfect agreement between LAME2 and LAME3 (linear weighting: $\boldsymbol{K_w}$ = 0.83, 95% CI

0.77 – 0.88; quadratic weighting: $\boldsymbol{K_w}$ = 0.89; 95% CI 0.85 – 0.93), versus only moderate to substantial agreement between LAME1 and LAME3 (linear: $\boldsymbol{K_w}$ = 0.50, 95% CI 0.43 – 0.57; quadratic: $\boldsymbol{K_w}$ = 0.64, 95% CI 0.58 – 0.70). The same patterns were found when evaluating reliability measures separately for the three largest farms, with better agreement between LAME2 and LAME3 (linear: $\boldsymbol{K_w}$ = 0.77 – 0.87; quadratic: $\boldsymbol{K_w}$ = 0.84 – 0.92), than when comparing LAME1 to LAME3 (linear: $\boldsymbol{K_w}$ = 0.36 – 0.61; quadratic: $\boldsymbol{K_w}$ = 0.47 – 0.73) for all three farms.

Table 2.7 Test attributes in relation to case definition and assessment frequency
Sensitivity, specificity, positive (PPV) and negative predictive value (NPV) were estimated with mixed logistic regression models (farm included as random intercept), using 1) LAME3 as gold standard when compared with LAME1 and LAME2[1]; and 2) ASSM1 as gold standard, when compared with ASSM2 and ASSM3[2]. Percent agreement was calculated through cross-tabulation.

	Sensitivity (95% CI)[3]	Specificity (95% CI)[3]	PPV (95% CI)[3]	NPV (95% CI)[3]	Percent agreement, %
Lameness definition[4]					
LAME1[5]	-	-	-	-	53
LAME2[5]	-	-	-	-	85
Assessment frequency[4]					
ASSM2	0.68 (0.62 - 0.74)	0.95 (0.92 - 0.97)	0.93 (0.89 - 0.96)	0.75 (0.69 - 0.80)	81
ASSM3	0.44 (0.39 - 0.51)	0.98 (0.95 – 0.99)	0.95 (0.92 – 0.97)	0.64 (0.58 – 0.70)	71

[1]LAME1 = ≥GS3 on ≥1 occasion; LAME2 = GS3 on ≥2 consecutive occasions, or ≥GS4 on ≥1 occasion; LAME3 = GS3 on ≥3 consecutive occasions, or ≥GS4 on ≥1 occasion.
[2]ASSM1 = weekly gait assessments; ASSM2 = gait assessments every 2 wk; ASSM3 = gait assessments every 3 wk; LAME2 was used to define lameness for all assessment intervals.
[3]Exact binomial confidence intervals.
[4]Total number of animals = 262.
[5]As the lameness definitions were nested, some test attributes could not be obtained due to complete data separation.

Farm incidence measures were higher when using LAME1 [average farm incidence = 81%; $t(5) = 10.8$, $p < 0.001$] and LAME2 [average farm incidence = 48%; $t(5) = 4.4$, $p = 0.007$] compared to LAME3 (average farm incidence = 35%).

Our findings demonstrate that assigning equal weight to occasional versus sequential GS3 scores introduces noise when identifying new cases of lameness (for example Figure 2.1a on page 52). It is possible that if we had performed locomotion scoring under more controlled conditions (i.e. not on commercial farms), the number of false positives would have been lower. Although we did not systematically assess movement when transitioning from lying to standing, we observed that heavy animals in late gestation often were visibly stiff directly after rising; this may have resulted in a higher rate of misclassification for the cows used in this study.

The low agreement between LAME1 and LAME3 suggests that lameness prevalence estimates contain uncertainty, and therefore should be viewed with caution. It is possible that the reported divergence in estimates of lameness prevalence between farmers and trained assessors (e.g. Espejo et al., 2006; Leach et al., 2010) is not due to assessors being more skilled in identifying mildly lame animals (e.g. Alawneh et al., 2012), but because farmers base their evaluations on multiple observations. In a qualitative study, Horseman et al. (2014) reported that many of the enrolled farmers (n = 12) were able to detect mild lameness, but rather referred to this as impaired mobility. Leach et al. (2012) reported that some lameness cases seem to self-cure, which may explain why farmers only consider LS4 and LS5 as ‘true’ lameness.

Even though LAME3 was used as gold standard in our analyses, it is possible that the duration of some true lameness cases might have been too short for the animals to be identified using this definition under the conditions of our study (weekly gait assessments). Given the high agreement and reliability between LAME2 and LAME3, we suggest that two consecutive GS3 scores is a reasonable cut-off for new cases of mild lameness in longitudinal research.

2.3.3 Relation between lameness definition and claw lesions

Table 2.8 on page 56 shows the sensitivity, specificity, PPV, NPV and percentage of agreement when LAME1, LAME2, and LAME3 compared with recorded trim findings. The high sensitivity values for LAME1 suggest that most animals with claw lesions at trimming had at least a brief period of altered gait during the dry period. However, the low specificity and PPV indicate that many of the animals identified as lame using the LAME1 criterion had no lesions at trimming. When LAME2 and LAME3 definitions were used the number of false positives decreased, leading to higher specificity and percent agreement for both non-infectious and infectious lesions. When comparing LAME2 and LAME3, LAME2 had higher sensitivity (non-infectious: 0.89 vs. 0.64; infectious: 0.69 vs. 0.64), and lower specificity (non-infectious: 0.62 vs. 0.71; infectious: 0.66 vs. 0.77). All case definitions had high NPV, indicating that cows that were never gait scored ≥3 were unlikely to have lesions at trimming.

Table 2.8 Test attributes in relation to claw lesions

Sensitivity, specificity, positive (PPV) and negative predictive value (NPV) for identification of animals with claw lesions using different lameness definitions[1] were estimated with mixed logistic regression models (farm included as random intercept). Percent agreement was calculated through cross-tabulation. Separate tests were performed for non-infectious[2], and infectious[3] claw lesions.

	Sensitivity (95% CI)[4]	Specificity (95% CI)[4]	PPV (95% CI)[4]	NPV (95% CI)[4]	Percent agreement, %
Non-infectious lesions[5]					
LAME1	0.90 (0.84 – 0.95)	0.21 (0.14 – 0.29)	0.08 (0.04 – 0.14)	0.96 (0.91 – 0.99)	26
LAME2	0.89 (0.82 – 0.94)	0.62 (0.53 – 0.71)	0.16 (0.10 – 0.24)	0.99 (0.95 – 1.00)	64
LAME3	0.64 (0.55 – 0.73)	0.71 (0.62 – 0.79)	0.16 (0.09 – 0.23)	0.96 (0.91 – 0.99)	70
Infectious lesions[5]					
LAME1	0.92 (0.86– 0.96)	0.24 (0.17 – 0.33)	0.05 (0.02 – 0.11)	0.98 (0.95 – 1.00)	39
LAME2	0.69 (0.60 – 0.77)	0.66 (0.56 – 0.74)	0.09 (0.04 – 0.15)	0.98 (0.94 – 1.00)	67
LAME3	0.64 (0.55 – 0.73)	0.77 (0.68 – 0.84)	0.12 (0.07 – 0.19)	0.98 (0.93 – 1.00)	74

[1]LAME1 = ≥GS3 on ≥1 occasion; LAME2 = GS3 on ≥2 consecutive occasions, or ≥GS4 on ≥1 occasion; LAME3 = GS3 on ≥3 consecutive occasions, or ≥GS4 on ≥1 occasion.
[2]Sole haemorrhage, sole ulcer, white line lesion, and toe ulcers.
[3]Digital dermatitis, and interdigital dermatitis.
[4]Exact binomial confidence intervals.
[5]Total number of animals = 117.

2.3.4 Effects of assessment frequency

The LAME2 criteria were used when evaluating the effect of assessment frequency. Fewer animals were categorised as becoming lame when assessment frequency decreased [ASSM1: n = 131 (50% of enrolled animals); ASSM2: n = 96 (37%); ASSM3: n = 62 (24%)]. The increasing difficulty in correctly identifying new lameness cases when assessment occurred less frequently is reflected in the higher sensitivity and negative predictive values for ASSM2 vs. ASSM3 when evaluated against weekly assessments (see Table 2.7 on page 53).

The weighted kappa values showed substantial agreement between ASSM1 and ASSM2 (linear: $\boldsymbol{K_w}$ = 0.68, 95% CI 0.60 – 0.76; quadratic: $\boldsymbol{K_w}$ = 0.75, 95% CI 0.67 – 0.82), and moderate agreement between ASSM1 and ASSM3 (linear: $\boldsymbol{K_w}$ = 0.48, 95% CI 0.39 – 0.57; quadratic: $\boldsymbol{K_w}$ = 0.55, 95% CI 0.45 – 0.65; Landis and Koch, 1977). Similar results were obtained when evaluating reliability measures for the three largest farms, with higher agreement between weekly and fortnightly assessments (linear: $\boldsymbol{K_w}$ = 0.63 – 0.78; quadratic: $\boldsymbol{K_w}$ = 0.73 – 0.85), than when comparing weekly assessments with assessments occurring every 3 wk (linear: $\boldsymbol{K_w}$ = 0.12 – 0.60; quadratic: $\boldsymbol{K_w}$ = 0.12 – 0.68).

A large percentage of animals categorised lame when assessed weekly was classified as sound when assessed every other (47% of mildly, and 12% of severely lame animals), and every third week (72% of mildly, and 33% of severely lame animals). Approximately 20% of the animals categorised as severely lame when assessed weekly were classified as mildly lame when assessed less frequently. Farm lameness incidence was higher when animals were scored every week (average farm incidence = 48%), compared to every other [average farm incidence = 36%; $t(5) = 3.1$, $p = 0.03$], and every third week [average farm incidence = 25%; $t(5) = 4.9$, $p = 0.004$].

2.3.5 Lameness duration

To explore why lameness classification differed between ASSM1, ASSM2, and ASSM3, the number of weeks an animal was categorised as lame was determined from ASSM1, using two consecutive scores of GS2 as a definition of cured. As the data collection ended at calving, lameness duration for cows classified as lame on the last assessment was unknown. Twelve percent of the animals lame for ≤2 wk were categorised as lame when censored; the corresponding value for animals lame ≥4 wk was 78%. Of the animals that changed category from lame to sound when assessed less frequently, 69% (ASSM2) and 42% (ASSM3) were lame for ≤2 wk, while 21% (ASSM2) and 49% (ASSM3) were lame for ≥4 wk.

Figure 2.2 shows a frequency histogram of number of weeks classified as lame for mildly and severely lame animals. The markedly bimodal distribution of lameness duration for mildly lame animals suggests that either a number of animals were incorrectly classified as lame when assessed weekly, or that this distribution represents two populations of animals with different underlying causes of lameness.

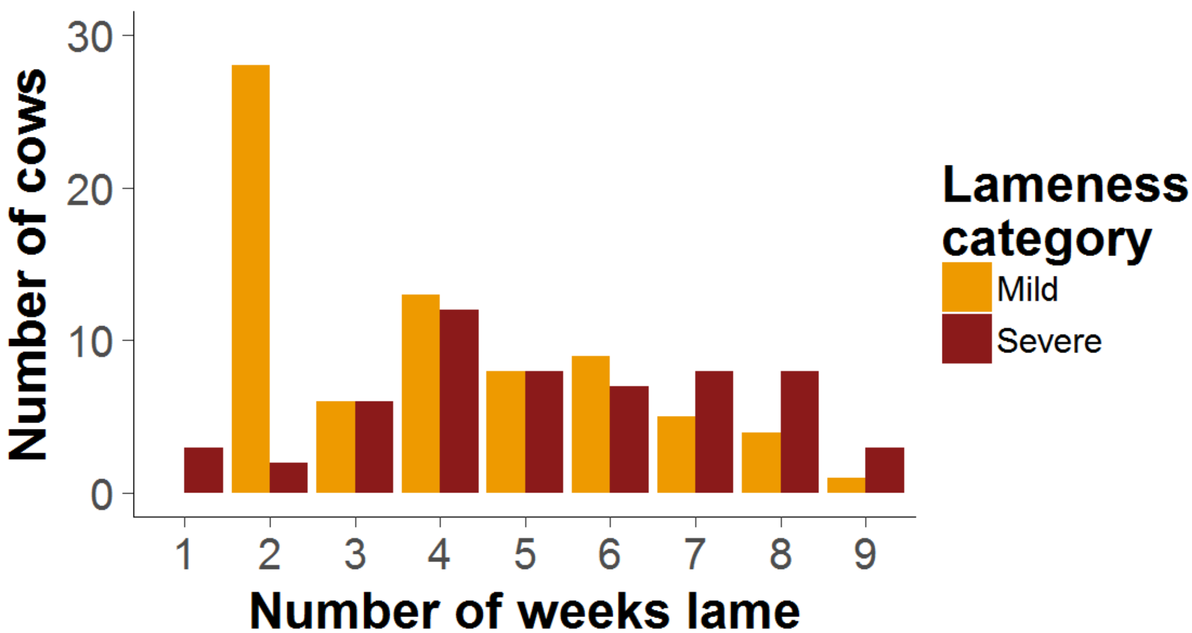

Figure 2.2 Number of weeks lame during the dry period
Frequency distribution of the number of weekly visits assessed as lame during the dry period, contrasting mildly and severely lame cows. To be considered mildly lame animals needed to be scored GS3 on ≥2 consecutive occasions, while severe lameness was defined as at least one score of ≥GS4.

In our study, 35% of the animals with infectious claw lesions (9 of 26 animals) scored ≥GS3 for ≤1 wk, while the same was true for 11% of the animals with non-infectious claw lesions (1 of 9 animals). Since lame cows were reported to the farmers after every visit, it is possible that treatment was initiated shortly after the onset of lameness, at least on some farms. Early treatment may have shortened the duration of readily treatable lameness cases, resulting in the bimodal distribution of lameness duration. Consistent with this idea, Whay et al. (1998) reported that animals lame due to interdigital phlegmon or digital dermatitis were all sound 28 d after treatment, while lame animals treated for sole ulcers, white line disease, or heel-erosion still had impaired gait. Multiple studies have reported that previous lameness events increase the risk of future lameness (e.g. Rowlands et al., 1986; Green et al., 2014; Randall et al., 2017), but so far the effect of previous lameness duration has not been evaluated. Before this relation is assessed, it would be premature to conclude that short lameness events are inconsequential for the animals.

In the current study lame animals often changed locomotion scores between assessments. For example, 89% of severely lame animals were scored GS3 at least once during the lameness event, and 22% of mildly lame and 28% of severely lame animals were occasionally scored GS2. These fluctuations resulted in some animals changing from lame to sound when assessed less frequently (ASSM2: 19% of all misclassified animals; ASSM3: 18%; see Figure 2.1b and Figure 2.1c on page 52 for examples). The number of animals presenting the locomotion patterns illustrated in Figure 2.1 are provided in Table 2.9 on page 60.

Based on the large number of cows identified as lame for ≥4 wk that were misclassified as sound when assessed every third week we suggest that locomotion should be evaluated at least fortnightly when conducting longitudinal research. If lameness events of short duration are of interest, assessment should occur even more frequently.

Table 2.9 Observed gait patterns
Descriptive statistics for gait patterns observed from weekly assessments during the dry period. How frequently the patterns occurred was evaluated for all animals, and also by contrasting animals remaining sound and animals becoming lame according to the LAME2 critera[1].

Defining gait pattern[2]	Animals with sequence, n	Prp of all animals (n = 260[3])	LAME2 lameness categories[1]: Prp of sound (n = 131)	LAME2 lameness categories[1]: Prp of lame (n = 130)
…2 – 2 – 2…[4]	47	0.18	0.36	NA
…2 – 3 – 2…[5]	35	0.13	0.27	NA
…2 – ≥3 – 2 – ≥3 – 2 – ≥3…[6]	13	0.05	0.03	0.07
…≥3 – ≥3 – 2 – ≥3 – ≥3…[7]	10	0.04	NA	0.08
…≥3 – ≥3 – 2 – 3 – 2 – 2… or …2 – 2 – 3 – 2 – ≥3 – ≥3…[8]	14	0.05	NA	0.11
…2 – 2 – 3 – 2 – 3 – 2 – 2… [9]	4	0.02	0.03	NA

[1]Criteria for lameness was ≥2 consecutive GS3 scores, or at least one score ≥GS4.
[2]Depicted GS vectors show the defining gait pattern, additional criteria for the individual gait patterns is described below. Additional assessment could been performed before or/and after the gait pattern of interest (indicated by …), and more than one pattern could be identified/animal.
[3]Two animals were removed from this analysis as they lacked their second locomotion assessment.
To fulfil the gait pattern criteria, animals
[4]were always scored ≤GS2;
[5]had one score of GS3 and were scored ≤GS2 both before and after this occasion;
[6]had ≥3 short (1 – 2 wk) bouts of ≥GS3, interrupted by short (1 – 2 wk) bouts of ≤GS2;
[7]were lame for ≥5 wk and had one GS2 score bounded by ≥2 scores of ≥GS3 on both sides;
[8]had one GS2 score between one GS3 score and multiple scores ≥GS3 in the beginning, or the end, of a lameness event;
[9]were sound and had one ambiguous short bout characterised by two GS3 scores interrupted by one GS2 score.

As the cows in our study were only followed during the dry period, our findings may not be generalizable to other parts of the lactation cycle. However, Reader et al. (2011) reported that lactating cows frequently changed locomotion scores between fortnightly gait assessments, suggesting that the same challenges of interpreting longitudinal locomotion data are present also after calving.

2.3.6 Implications for Farm Management

A practical limitation of the current study is that we assessed locomotion on a weekly basis. Although this is a quite frequent sampling regime for a longitudinal lameness study, it differs considerably from the daily (albeit unsystematic) assessments that farmers self-reported in a study conducted by Horseman et al. (2014). Such short assessment intervals may enable farmers to use information from multiple gait assessments to more reliably identify mildly lame animals, while still being able to provide treatment within a reasonable time period. Further studies should evaluate what case definition would be most useful when cows can be assessed multiple times per day. As it is unlikely that animals with severely impaired gait are actually sound, examination and treatment of cows that easily can be identified as lame should be performed as soon as possible after first detection (Leach et al., 2012).

Some animal groups are not as regularly observed walking during daily work tasks (e.g. dry cows), and we suggest that these animals should be evaluated for lameness at least every other wk. This procedure may also be valuable in dairy systems with less intensive everyday human-animal contact (e.g. robotic farms). Given the effort required to gait scores cows this frequently, we urge work on the development of automated locomotion scoring systems suitable for use on commercial farms.

2.4 Conclusions

Estimates of lameness incidence increased when a less stringent lameness definition was used, and when assessment frequency increased. Our results suggest that occasional observations of GS3 should not be used as criterion for mild lameness in longitudinal studies. As assessment frequency decreased, it was increasingly difficult to identify both mildly and severely lame animals. We suggest that gait assessment should be performed at least every other week to generate reliable estimates of lameness incidence.

Chapter 3: Standing behaviour during the transition period and sole horn lesions

3.1 Introduction

Previous research has found that cows that spend more time standing are more likely to become lame (Galindo and Broom, 2000). This association may be due to the positive relationship between impaired gait and severe sole lesions (Flower and Weary, 2006; Chapinal et al., 2009), as animals with increased standing time are more likely to develop sole lesions (Singh et al., 1993; Galindo and Broom, 2000).

The transition period, i.e. from 3 wk before to 3 wk after calving, may be particularly important for claw health. The laxity increases in the hoof's supportive structure during this time, which is believed to increase the risk of mechanical damage (Tarlton et al., 2002; Knott et al., 2007). Thinning of the digital cushion reduces its shock absorbing capacity, which also may increase the risk of claw horn lesions (Räber et al., 2004). Newsome et al. (2017b) reported that animals with little soft tissue between the sole horn and the pedal bone had increased risk of future claw horn lesions. The thickness of the soft tissue between the pedal bone and the sole is reduced in animals with low BCS, perhaps as a result of fat mobilization from the digital cushion (Bicalho et al., 2009). However, Newsome et al. (2017a) found that the sole soft tissue was thinnest the week after calving, further supporting that the risk of claw damage is increased during the transition period.

Multiple studies have shown that standing behaviour during the transition period differs between animals that continue to have good claw health and those that develop severe sole lesions after calving (Chapinal et al., 2009b; Proudfoot et al., 2010). However, this research was performed under controlled conditions on a university research farm, limiting the extent to which results can be generalised.

When dairy cows are forced to stand, behaviours such as weight shifting and leg stomping are performed more frequently the longer the animal has been standing (Cooper et al., 2007; Krebs et al., 2011). These behaviours suggest that long standing bouts also may increase mechanical strain on the claw, leading to a higher risk of sole lesions. The relationship between standing bout duration and postpartum sole lesions has so far only been assessed for average bout duration (Proudfoot et al., 2010), but it may be more biologically relevant to evaluate the effect of particularly long standing bouts rather than the average bout duration.

Here we report the results of the first prospective longitudinal study evaluating what aspects of standing behaviour during the transition period that were related to the development of sole lesions later in lactation on commercial dairy farms. We hypothesised that dairy cows with increased standing time, and long standing bouts, would be at higher risk of developing sole lesions during the early lactation.

3.2 Material and methods

3.2.1 Farm selection

This study was approved by the Animal Care Committee at the University of British Columbia (protocol A15-0084). A convenience sample of eight commercial dairy farms in the Lower Fraser Valley region (BC, Canada) were visited every other week between May 2017 and January 2018. The farms were recruited by partnering with a local claw trimming company. Farms were eligible for enrolment if the herd size was ≥160 lactating animals, dry cows and pre-calving heifers were kept separately from lactating cows, and animals were predominately housed in free-stall pens (the use of deep-bedded open packs for a short period in the end of the dry period was acceptable). Additional prerequisites were that the farmers used herd management software with electronically extractable data, and that the farms recorded calving date and milk yield for individual animals. Farms were contacted by phone based on proximity to the UBC Dairy Education and Research Centre (Agassiz, BC, Canada). Of 13 contacted farms, nine agreed to participate in the study; one farm was excluded shortly after enrolment because the configuration of the dry pens impeded data collection.

During the initial farm visit a structured interview was conducted with the owner or manager to collect information about farm characteristics (see Table 3.1 on page 66) and claw health management (see Table 3.2 on page 67). On the same occasion, an environmental assessment was performed in the pre- and postpartum pens, walkways, waiting area for milking, and parlour (see Table 3.3 on page 68).

3.2.2 Sample size

A power analysis, based on reported sole lesion prevalence for primiparous animals in peak lactation (64%; Capion et al., 2009; 70%; Randall et al., 2016), and assuming that this animal group was unlikely to have experienced severe claw horn lesions previously (Leach et al., 1997) was undertaken. A 20% difference in animals developing sole lesions was considered biologically relevant (assumed prevalence of sole lesions for animals with standing time below median: 55%; above median: 75%). The analysis ($\alpha = 0.05$; $1-\beta = 0.8$) resulted in an estimate of 94 animals required in each group. Due to the clustering of data within farms, this estimate was considered conservative, so we set as target to enrol 300 cows.

Table 3.1 Farm characteristics and management on the enrolled farms
Eight dairy farms in the lower Fraser Valley region of British Columbia, Canada, visited every 2 wk from May 2017 to January 2018.

Farm	A	B	C	D	E	F	G	H
Herd size[1]	185	510	540	310	330	290	250	610
Rounded 305-d milk yield	12,700	12,800	12,900	10,500	9,100	12,200	12,800	13,000
Breed	Holstein	Holstein	Holstein	Holstein	Mixed[2]	Holstein	Holstein	Holstein
Geographical farm sites	1	3[3]	1	2[4]	1	1	1	2[4]
Milkings/d	2	3	2	2	2	2	3	2-3[5]
Feedings/d								
Dry animals	1	1	1	1	0.5[6]	1	1	1
Lactating animals	1	1	1	2	1	2	1	1
Cows/feed space[7]								
Dry pens	0.8	0.9	1.0	0.8	1.2	0.8	0.9	0.7
Lactating pens	1.0	1.0	0.9	1.0	1.1	1.3	0.9	0.5
Cows/lying space[8]								
Dry pens	1.0	0.7	0.8	0.8	1.0	0.9	0.9	0.8
Lactating pens	1.0	0.8	0.9	1.0	1.1	1.0	1.0	0.8
Pen-changes[9]	5	5	5	5	4-5[10]	6	5	6
Introduction to main herd[11]	-3	-3	-2	-8	-6	0	-8	-5
First parity lactation pen	no	yes	yes	yes	yes	no	yes	yes[12]

[1]Sum of dry and lactating animals, pregnant heifers are not included.
[2]Animals cross-bred with Holstein, Ayrshire, and Jersey.
[3]Dry animals were kept on a separate farm in the same district, and the heifers were kept on a dry-lot in another district.
[4]Heifers were kept on a dry-lot in another district.
[5]Farm changed to three milkings/d midway through the study period.
[6]Dry cows fed every other day.
[7]One feed space was defined as either one head-lock, or 60 alternatively 76 cm linear feed space for lactating and dry cows, respectively.
[8]One lying space was defined as either one free-stall, or 11m^2 in the open pack lying area.
[9]Number of pen-changes from dry-off to early lactation.
[10]Primiparous cows = four pen-changes, multiparous cows = five pen-changes.
[11]Time-point when first-calving animals were first mixed with dry or lactating multiparous cows, measured as week in relation to calving.
[12]Until confirmed pregnant, then mixed with multiparous cows.

Table 3.2 Lameness prevalence and claw health management on the enrolled farms
Eight dairy farms in the lower Fraser Valley region of British Columbia, Canada, visited every 2 wk from May 2017 to January 2018.

Farm	A	B	C	D	E	F	G	H
Lameness prevalence[1]	42 (7)	27 (3)	55 (25)	32 (3)	30 (7)	32 (11)	21 (3)	25 (3)
Footbath/wk	2	1	2	4	1	0	3	2
Solution[2]	F	F	F/Zn[3]	Cu/Zn[4]	F	-	F	F/Zn[5]
Trimmings/lactation	1-2	2	2	3	ad hoc	1	2	1
Locomotion assessment								
Lactating cows	Weekly[6]	Daily[7]	Daily[7]	Daily[7]	Daily[7]	Daily[8]	Daily[7]	Daily[7]
Dry cows	Weekly[6]	-	-	2 t/wk	-	Daily[8]	-	-
Management acute lameness[9]								
Mild	TL	TL	-	TL	TL	TL	O	O
Severe	A, O	A, O, C, P	A	A, O, C, P	O	O	O	O, C
Follow-up[10]	P	L, TR	L, TR	L, TR	L, TR	L, X	OR	L, TR

[1]Lactating herd prevalence (%) of clinical lameness at the initial farm visit, values within brackets are percent animals with ≥GS4.
[2]F = formaldehyde; Zn = zinc sulphate; Cu = copper sulphate.
[3]Formaldehyde used during summer months, and acidified zinc sulphate during winter months.
[4]Copper and zinc sulphate used on alternating occasions.
[5]Formaldehyde and zinc sulphate used on alternating occasions.
[6]Assessment performed during scheduled occasions set aside for locomotion scoring.
[7]Assessment performed during milking.
[8]Assessment performed during pen walks for identification of unhealthy animals and animals in heat.
[9]A = acute trim (veterinarian or trimmer); C = moved close to parlour; O = own acute trim; P = moved to open pack; TL = put on trim list for next trimming visit.
[10]L = locomotion reassessed to evaluate treatment effect; P = moved to open pack; OR = recheck performed by farmer; TR = recheck by trimmer next ordinary visit; X = cull animals with poor treatment effect.

Table 3.3 Environmental assessment on the enrolled farms

Eight dairy farms in the lower Fraser Valley region of British Columbia, Canada, visited every 2 wk from May 2017 to January 2018.

Farm	A	B	C	D	E	F	G	H
Pen lay-out								
Far-off pens	free-stall	free-stall	free-stall	free-stall	free-stall	free-stall	free-stall	free-stall
Close-up pens	open pack	open pack	free-stall	open pack	free-stall	free-stall	free-stall	free-stall[1]
Lactating pens	free-stall	free-stall	free-stall	free-stall[2]	free-stall	free-stall	free-stall	free-stall
Manure handling	scraper	scraper	flush	tractor[3]	robot	scraper	scraper	scraper
Alley cleanliness[4]	0 (0-0)	1 (0-2)	0.5 (0-1)	1 (0-2)	0 (0-1)	0 (0-1)	1 (0-1)	0 (0-0)
Flooring[5]								
Far-off pens	concrete	concrete	concrete	concrete	slats[6]	rubber	concrete	concrete
Close-up pens	concrete	concrete	concrete	concrete	slats[6]	rubber	concrete	concrete
Lactating pens	concrete	concrete	concrete[7]	concrete	slats[6]	concrete	concrete	concrete
Walk-ways	concrete	concrete	concrete	concrete	slats[6]	concrete	concrete	concrete
Waiting area	concrete	concrete	concrete	concrete	concrete	concrete	rubber	concrete
Parlour	concrete	concrete	rubber	concrete	rubber	concrete	rubber	rubber
Parlour type	herring-bone	rotary	parallel	herring-bone	parallel	parallel	parallel	parallel
Meter to parlour[8]	31-50	6-69	33-54	0-17	4-36	7-98	-	11-97

[1]Animals moved to deep-bedded open pack 7 d before expected calving.
[2]Fresh cows housed on open pack for the first 3 – 5 d postpartum.
[3]Scrapers present in the first parity lactation pen.
[4]Alley cleanliness was measured in the feed alley; 0 = ≤0.5 cm; 1 = 0.5 – 1 cm; 2 = 1 – 3 cm; 3 = >3 cm manure depth. On average 39 pen assessments were performed/farm, values represent median (interquartile range).
[5]All flooring was solid unless indicated.
[6]Concrete slats.
[7]Rubber flooring was present by the feed-bunk.
[8]Distance from the pen-gate to the entrance of the parlour waiting area.

3.2.3 Study design

Based on previous research, a causal diagram was used to identify cow-level variables affecting the risk of claw horn lesions (Figure 3.1). Only animals with complete trimming records were eligible for enrolment; on one farm this excluded animals with parity 5 and higher. Animals with no record of severe claw horn lesion (sole ulcer, thin soles, toe ulcer, periopole ulcer, or white line disease) and sound 4 to 8 wk before expected calving were enrolled on an on-going basis. The animals were assessed every other week until approximately 8 to 12 wk postpartum, when they were claw trimmed as part of the study. The trim period was chosen to allow for sole horn lesions developing during the transition period to become visible on the sole surface (Kempson and Logue, 1993). Parity, expected and actual calving date were retrieved from the herd management software.

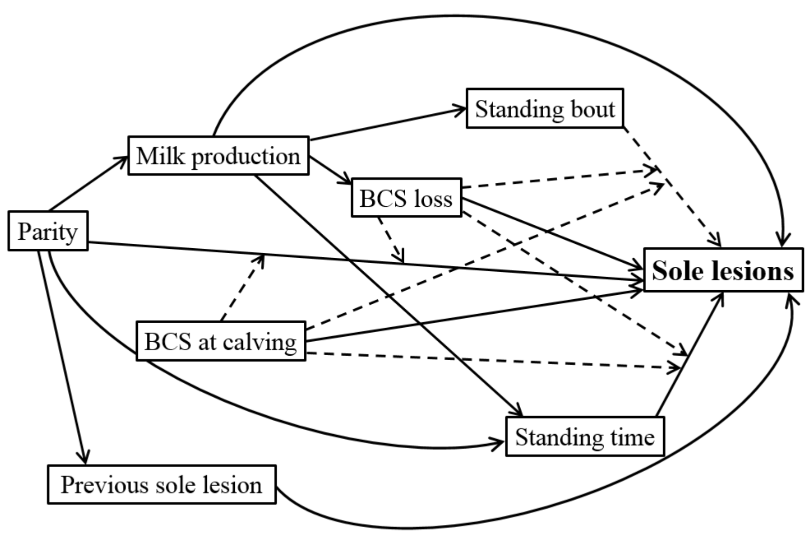

Figure 3.1 Causal diagram for sole lesion risk factors
Proposed cow-level factors affecting the risk of sole horn lesions. Line type indicates the level of current evidence: 1) support from peer-reviewed literature (solid line), 2) proposed by the present study (dashed line). Arrows between boxes illustrate direct effects, and arrows touching arrows illustrate moderating effects.

3.2.4 Standing behaviour

To measure standing behaviour, enrolled animals were fitted with data loggers (HOBO Pendant G Acceleration Data Logger; Onset Computer Corporation, MA, US) on one of their rear legs, approximately 7 – 10 cm above the fetlock. Standing behaviour was recorded from 4 to 5 wk before expected calving date until 2 to 3 wk postpartum, and loggers were changed every other week. Loggers were attached so that the y-axis pointed upwards, and were set to record *g*-force acceleration for the y-axis once/min.

Logger data were downloaded using HOBOware (Onset Computer Corporation, MA, US) and raw *g*-force values were exported as CSV files, which were imported into R version 3.4.4 (R Core Team, 2018; RStudio Team, 2016) for further analyses. To ensure that all *g*-force values were positive, a value of 3.2 was first added to the raw data values. Then a previously validated cut-off (**2.55**; Ledgerwood et al., 2010) was used to convert the y-axis values to binary values, with 0 representing standing (values <2.55), and 1 representing lying (values ≥ 2.55) for each cow. The registered *g*-force values represent the orientation of the rear leg at the moment of recording, which means that e.g. lifting the leg to a horizontal position would result in values indicative of lying being recorded for standing animals. To reduce the number of erroneous data points, a filter was applied to transform single and double recordings of ‘1’ in a string of ‘0’s to ‘0’, and vice versa (2-min filter; Ledgerwood et al., 2010). The filtered data were summarised to determine standing time (min) and the duration of the longest standing bout (min) for each cow and day. Days when data loggers were attached, changed, or removed were excluded from the data set.

3.2.5 Locomotion

Before data collection started two observers with previous experience in gait scoring (GS) were trained to align their ratings using an ordinal scale from 1 to 5 (GS1 = smooth and fluid movement, GS2 = imperfect locomotion, GS3 = compromised locomotion, GS4 = obviously diminished ability to move freely, and GS5 = severely restricted ability to move; Flower and Weary, 2006). Details about the training methodology can be found in section 2.2.3 on page 44.

To estimate lameness prevalence on the enrolled farms, the locomotion of all lactating animals was assessed as animals exited the parlour during the initial visit on each farm (see Table 3.2 on page 67). Enrolled animals were locomotion scored on each visit (every 2 wk) from enrolment until the first visit after trimming. Assessment was performed during pen walks where unrestricted animals walked on concrete while being observed directly and obliquely from behind by one of the observers. Information about animals with ≥GS3 was given to the farms after every visit (except on those farms that specifically requested only information about ≥GS4), and treatment was performed at the discretion of the farm manager.

3.2.6 Claw lesions

Postpartum trimming was performed by one of two professional trimmers, belonging to the same trimming company through which the farms were recruited. Claw lesions were recorded in accordance with the company's normal routines, and identified lesions were registered per claw according to Alberta Dairy Hoof Health Project's Lesion Severity Scoring Guide (*dairyhoofhealth.info/Lesion-Severity-Guide-v0.7.pdf*) using the Hoof Supervisor System™ software (KS Dairy Consulting, Inc., Dresser, WI, USA). Findings registered at trimming were obtained from transcripts. A cow was considered affected with a lesion if it was found on at least one claw, and the severity of the most serious injury was noted per lesion type. If more than one type of lesion was present at trimming, the animal was considered affected with all lesion types identified.

3.2.7 BCS

BCS was measured in 0.5 point increments using a ordinal scale from 1 to 5 (1 = very thin, 2 = thin, 3 = good condition, 4 = over conditioned, 5 = obese; Ferguson et al., 1994) by one of four observers, jointly trained before the start of data collection. Inter-observer reliability was evaluated from 54 cows, scored by all observers on the same day on one of the participating farms. While assessing the cows, the observers did not share information about what scores were assigned; the minimum score assigned was 2 and the maximum was 5. Intra-class correlation (**ICC**) was used to evaluate the reliability, as it allows for evaluation of more than two observers of ordinal data. As the observers were non-randomly selected and it was important that the scores were similar in absolute value, the used ICC variant was specified as two-way and absolute agreement (Hallgren, 2012). The ICC generally can take values from -1 (perfect disagreement) to 1 (perfect agreement), with 0 indicating agreement no better than chance; using the framework of Cicchetti (1994), the ICC obtained for the four observers (0.81; 95% CI: 0.73 – 0.87) indicated good to excellent agreement for BCS assessment.

BCS measurements were taken at approximately 8, 4 and 2 wk before estimated calving, at calving, and 2, 4 and 8 wk postpartum, allowing for the fortnightly visit intervals. Change in BCS from the dry period to peak lactation was calculated by subtracting the highest BCS score during the dry period from the lowest recorded BCS after calving.

3.2.8 Milk production

On a visit occurring between 50 – 100 d postpartum, DIM and 24-h uncorrected milk production (kg) were recorded through the farms' herd management software programs. One farm used monthly recordings to measure milk production, so for these cows we used data from one milk recording occurring during the same period.

3.2.9 Data handling and statistical analyses

When assessing the animals, data was recorded on paper and later transcribed into an on-line electronic repository. The transcription error rate was assessed in 5% of the full data set, with 0.008% of the measurements being incorrectly entered. When mistakes were discovered, the entries were corrected. For further data handling and analyses the data was imported as csv-files into R 3.4.4 (R Core Team, 2018; RStudio Team, 2016; dplyr, Wickham et al., 2017; tidyr, Wickham and Henry, 2018). Data visualization was performed with the ggplot2 package (Wickham, 2009).

3.2.9.1 Data handling

Animals were categorised as becoming lame if they were scored GS3 on ≥2 consecutive visits or ≥GS4 at least once (see Chapter 2 for an evaluation of the lameness definition), with ≥GS4 being considered severely lame. Cows that became lame before calving were not included in postpartum analyses, to further reduce the risk of including animals with previous claw problems in the data set. When analysing risk factors for sole lesions we used animals without any claw lesion as the reference category, animals with other types of claw lesions were excluded from these analyses.

Standing behaviours were transformed from min/d to h/d and summarised per cow by the following periods: before (d -14 to d -2), around (d -1 to d 1), and after (d 2 to d 14) calving. Average daily standing time, average daily longest standing bout (average length of the longest daily standing bout per period), and the longest standing bout per period were summarised by cow. The periods were tested separately to assess if the effects of prolonged standing differed between periods. Animals with less than 5 d of standing behaviour in the pre- or postpartum period were removed from the analyses of respective period. The effect of change in average daily standing time and average daily longest standing bout from before to after calving were also analysed.

BCS at calving was estimated using the average score from the last two visits before calving. The animals were dichotomised as BCS <3.25 vs. BCS ≥3.25 at calving. Based on the results of Lim et al. (2015), who reported that animals loosing ≥0.75 BCS score in early lactation had increased lameness risk, cows loosing ≤0.5 BCS points were considered loosing no to little BCS in early lactation, while animals loosing ≥1.0 points were considered having substantial BCS loss.

3.2.9.2 Statistical analysis

Cow was considered experimental unit in all analyses. Extreme data values (≥3 SD above or below the mean) were evaluated before analysing the data. Erroneous data due to malfunctioning loggers were removed and incorrect data entries were corrected.

Pre- and postpartum lameness incidence rate was estimated separately for primiparous and multiparous animals. Cows with <3 assessments were not included in the analysis, as the criteria for lameness could not be met by these animals. Number of cow-weeks at risk was calculated using the exact method (Dahoo et al., 2009), assuming that lameness events occurred midway between visits. Animals becoming lame in a previous period were no longer considered at risk. To evaluate when the first postpartum lameness event occurred most frequently, the postpartum period was split into first, second and third month after calving. Cows with <2 assessments after calving were excluded from postpartum incidence analysis. Crude sample odds ratio (**OR**) for becoming clinically and severely lame after calving, depending on what type of claw lesion was found at trimming (no lesion, horn lesions or infectious lesions) was calculated from the raw data. No statistical inference is provided for the descriptive analyses as we did not have priori hypotheses for these results.

To evaluate the relationship between standing behaviour and sole lesions, mixed effects logistic regression models were fitted with maximum likelihood using the glmer function in the lme4 package (Bates et al., 2015), and. Separate models were fitted for the periods before, around, and after calving, and also for change in standing behaviour from the pre- to postpartum period. Likelihood ratio tests were used to compare subsets of models, to assess if additional terms improved model fit. Farm was included in the models as random intercept. Random slopes of average daily standing time and average daily longest standing bout were not retained as they did not improve model fit. Predictor distribution, and the relationship between predictors were assessed graphically (package GGally; Schloerke et al., 2018). Pearson's correlation coefficient was calculated for all linearly related continuous predictors. Average daily longest standing bout and the longest standing bout of the period were strongly correlated for all periods ($r \geq 0.8$; $n \geq 259$), so only average daily longest standing bout was included in the models. The Pearson correlation coefficient between average daily standing time and average daily longest standing bout was lower for all periods ($r < 0.7$; $n \geq 259$), so initially the behaviours were evaluated in the same models. This resulted in considerably altered estimates (up to 85% decrease in slope) and increased uncertainty (approximately 30% increase in standard error) for both variables, so separate models with the same covariates were fitted for the two standing behaviours. The same was found when analysing change in average daily standing time and change in average longest standing bout, so separate models were fitted also for change in standing behaviour.

Cow-level covariates included in the full models (based on the causal diagram) were parity, estimated milk production at 50-100 DIM, BCS at calving and BCS loss in early lactation. Not all animals were trimmed within the target period of 8 to 12 wk postpartum, so DIM at trimming was included as covariate. Continuous predictors were centred at their means. Biologically plausible two-way interactions (standing behaviours x BCS at calving, standing behaviours x parity, parity x BCS at calving, and parity x BCS loss) were tested in separate models as the full model did not converge when interactions were included, and significant interactions were evaluated graphically. Parity x BCS loss, and parity x BCS at calving did not converge due to insufficient data, and were evaluated by cross-tabulation. Complete data separation was present for parity x BCS loss, as all multiparous cows with sole lesions lost ≥1 BCS in early lactation. Cross-tabulation did not indicate an interaction between parity x BCS at calving. As interactions could not be tested in the full models statistical inferences for these terms are not reported, and the results from the final models include main effects only. Model fit was checked by inspecting residuals at each level. Influential data points were removed and models refitted to evaluate if this changed the model coefficients.

3.3 Results

3.3.1 Overview of the data set

A total of 513 animals were initially assessed for enrolment, with a median of 55.5 animals (range: 19 – 70) enrolled cows/farm. See Figure 3.2 for the number of animals that was assessed, enrolled, and completed the study. Of the enrolled animals, 13 completely lacked standing behaviour either because no data logger was available before calving (n = 7) or because of malfunctioning loggers (n = 6). Fifty animals left the study before trimming: one developed

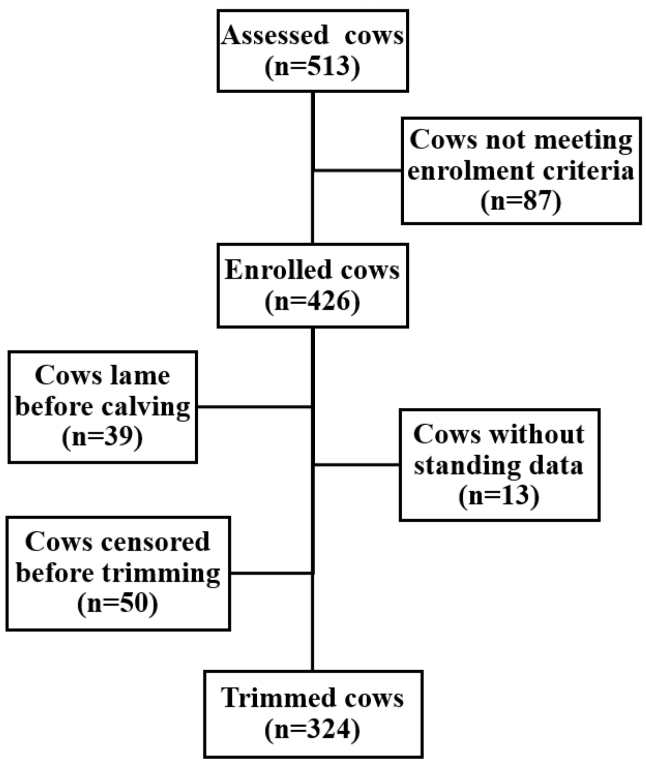

Figure 3.2 Number of cows enrolled in and completing the study
Animals with no previous severe claw horn lesion and sound at first assessment 4 to 8 wk prepartum were locomotion scored every other week from the dry period until trimming at 11±2 wk postpartum. Cows becoming lame during the dry period, or lacking measures of standing behaviour were excluded from sole lesion analyses. Censored animals were removed from the herd based on decisions made by the farm manager.

downer cow syndrome after calving, six either aborted or calved so early that standing behaviour was not recorded, one had an unknown due date, 15 were culled, 16 were sold, nine died on-farm (five from unknown causes, two from mastitis, one from ketosis, and one from abomasal displacement), one could not be locomotion scored for >2 months postpartum as it was equipped with hobbles after a milking related accident, and one was too dangerous to handle. An additional 39 animals became lame before calving and were removed from the postpartum analyses.

The number of animals in lactation number 0, 1, and 2+ was 189 (44%), 124 (29%), and 111 (26%) at enrolment (two animals had unknown parity), while 149 (46%), 100 (31%), and 75 (23%) cows completed the study, respectively. For animals completing the study the median BCS at calving was 3.25 [interquartile range (**IQR**): 3.00 – 3.75] for primiparous and 3.50 (3.25 – 3.75) for multiparous animals. Only 11 (3.4%) of the animals had a BCS <3.00 at calving. Both parity groups lost a median of 1.0 BCS point in early lactation, with an IQR of -0.5 to -1.0 for first-calving and -0.5 to -1.5 for multiparous animals. Thirty percent of the primiparous animals had BCS <3.25 at calving, and 52% lost ≥1.0 BCS score in early lactation, the corresponding values for older cows were 17% and 72%, respectively. Mean±SD milk yield was 31.7±5.9 for first-calving animals and 43.7±7.9 for multiparous cows; three animals lacked milk yield data.

3.3.2 Lameness incidence

The median number of locomotion scores/animal was 11 (IQR: 9 – 12). On 35 occasions we could not perform locomotion assessment (0.8% of all assessments); on two occasions the cows calved during the visit, on 12 occasions the animals could not be found in their home-pen, and on one visit the farmer did not allow assessment of fresh cows because of high ambient temperature (meaning that 21 cows could not be scored on that date). Thirty-one animals lacked one locomotion score, and two cows lacked two scores.

Figure 3.3 illustrates the incidence rate of lameness before and after calving. The distribution differed between primiparous and multiparous cows, with older animals generally having higher incidence. Lameness incidence was lower during the dry period for both parity groups. The postpartum incidence of lameness remained stable at 8 – 9 cases/100 cows per month over the first 12 wk of lactation for the primiparous animals. The lameness incidence for multiparous cows was initially higher, but dropped to the level of primiparous cows 3 mo after calving.

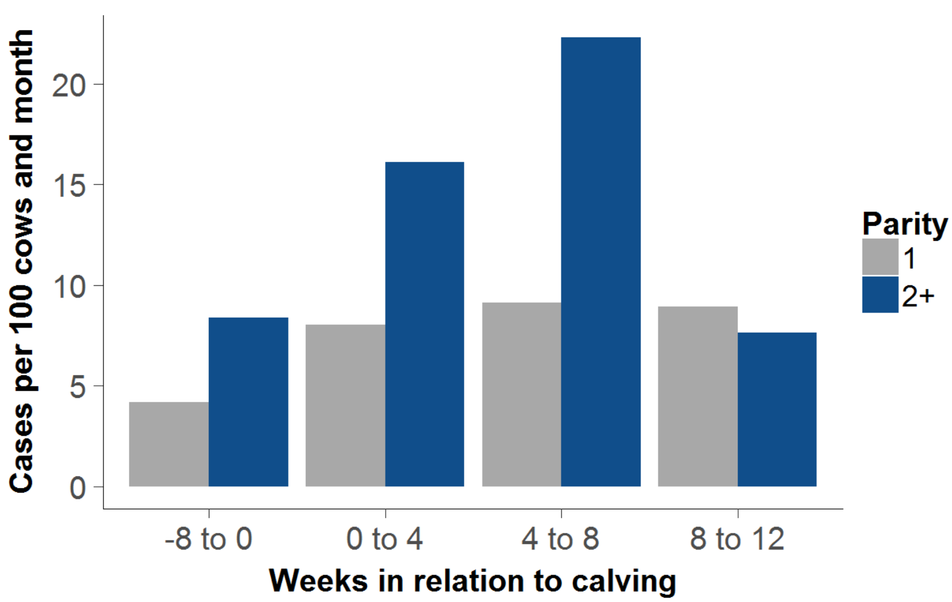

Figure 3.3 Lameness incidence during the dry period and early lactation
Primiparous and multiparous cows were locomotion scored every other week on eight farms in the lower Fraser Valley region of British Columbia, Canada. Animals becoming lame in a previous period were no longer considered at risk.

3.3.3 Claw lesion prevalence

Of the enrolled animals that remained sound until calving, 324 were trimmed at 11±2 wk (mean±SD) postpartum. Sixty-two percent of the animals were trimmed within the target period 8 to 12 wk postpartum. Primiparous animals were trimmed at a median 79 DIM (IQR: 69.0 – 91), and older cows at 77 DIM (67.0 – 87.5).

The claw lesion prevalence was 4.3% for sole haemorrhages, 3.7% for sole ulcers, 0.9% for white line lesions, 0.9% for thin soles, 10.2% for digital dermatitis, 4.9% for interdigital dermatitis, and 0.3% for foot rot. Seventy-six percent of the animals had no registered claw lesions, and the proportion of animals with lesions was similar between parity groups (parity 1 = 23.5%; parity 2+ = 24.6%). It was numerically more common for first calving animals to have sole haemorrhages (6.0% vs. 2.9%), and for older animals to have sole ulcers (5.1% vs. 2.0%).

3.3.4 Postpartum gait in relation to claw lesions

Figure 3.4 on page 81 shows the relationship between postpartum lameness status and the type of claw lesion found at trimming. Compared to animals without claw lesions, animals with claw horn lesions (sole lesions, white line lesions and thin soles) had 2.5 times higher sample odds of becoming lame after calving, and 11.7 times higher sample odds of becoming severely lame. When we contrasted animals with claw horn lesions to animals with infectious lesions (digital dermatitis, interdigital dermatitis, and foot rot), the corresponding values were 0.8 for becoming lame and 2.3 for becoming severely lame. Sixty-nine percent of the cows that had severe claw horn lesions (sole ulcers and white line disease; n = 13) became lame after calving, and 46% became severely lame. Conversely, 28% of the animals with milder horn lesions (n = 18) became lame, and 11% became severely lame.

Of the animals that became lame after calving, 33%, 57%, 90% and 98% of animals with no lesion at trimming became lame within 2, 4, 8, and 12 wk after calving, respectively. The corresponding values for lame animals with horn and infectious lesions were 21%, 57%, 86% and 100%, and 13%, 25%, 71% and 96%, respectively. Median duration of lameness at the end of the study period was 8 wk (IQR: 6.0 – 10.0) for lame cows with no lesions, 10 wk (IQR: 6.5 – 14.0) for lame animals with horn lesions, and 6 wk (IQR: 4.0 – 8.0) for cows with infectious lesions. Censoring occurred at 12.5±3.0, 13.1±2.5 and 13.0±2.3 wk postpartum for animals with no, horn and infectious lesions, respectively. Seventy-six percent of the animals that became lame postpartum were still lame in the end of the study period.

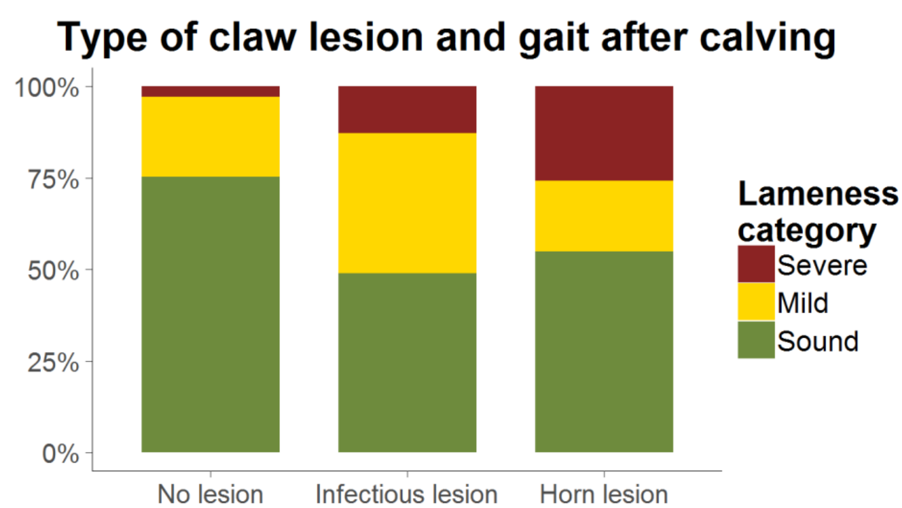

Figure 3.4 Proportion of animals remaining sound and becoming lame postpartum
The animals were grouped by the type of claw lesions identified at trimming 11±2 wk postpartum [claw horn lesions (n = 31); infectious lesions (n = 47); no lesion found (n = 246)]. No animal had both horn and infectious claw lesions.

3.3.5 Standing behaviour

Of the 324 animals with trim records, 316, 309 and 310 had standing behaviour data before (d -14 to d -2), around (d -1 to d 1), and after calving (d 2 to d 14), respectively. Average daily standing time was normally distributed, with a mean±SD of 12.1±1.6 h/d, 14.3±2.2 h/d and 13.8±1.7 h/d for the three periods, respectively. The distributions for average daily longest standing bout and the longest standing bout of the period were right skewed. Average daily longest standing bout had a median of 3.6 h/d (IQR: 3.0 – 4.3; range: 1.7 – 12.1), 3.9 h/d (IQR: 3.1 – 4.8; range: 1.3 – 11.5) and 3.7 h/d (IQR: 3.2 – 4.4; range: 1.5 – 11.7) before, around, and after calving, respectively. The median longest standing bout of the period were 5.5 h (IQR: 4.3 – 7.3; range: 2.4 – 21.7), 4.9 h (IQR: 3.7 – 6.6; range: 2.1 – 18.1) and 5.9 h (IQR: 4.8 – 7.3; range: 1.9 – 26.3), respectively.

Standing data were available both pre- and postpartum for 302 animals; the mean±SD change in average daily standing time was 1.8±1.8 h/d, while the change in average longest daily standing bout was 0.1±1.3 h/d. We found a moderate and positive linear correlation between change in standing time and change in longest standing bout ($r_{pearson} = 0.65$; n = 302).

3.3.6 Temporal association between standing behaviour and sole lesions

For analyses of sole lesions, standing data were available for 262, 253 and 256 animals before, around and after calving, respectively, while 250 cows had data for change in standing behaviour. No association was found between standing behaviour and sole lesions before or around calving (see Table 3.4 on page 83). During the postpartum period, both an increase in average daily standing time and average longest standing bout were related to increased odds of sole lesions (Table 3.4; Figure 3.5 on page 84). Graphical evaluation of significant interactions (see Figure 3.6 on page 86) suggests that the relationship between average daily standing time and sole lesions was stronger for multiparous cows. Random slopes for average daily standing time, and average daily longest standing bout did not improve model fit, which indicate that the relationship was consistent on the farms enrolled in our study. An increase in average daily standing time from before to after calving was not related to sole lesions (Table 3.4), but graphical evidence suggests that this was due to one highly influential data point (see Figure 3.5 on page 84). A 1-h increase in average daily longest standing bout increased the odds of sole lesions 1.5 times (Table 3.4).

Table 3.4 Association between standing behaviour and sole lesions
Results from multiple mixed-effects logistic regression models[1] used to evaluate the effect of standing behaviour in late gestation and early lactation on the probability of sole lesions at 11±2 wk postpartum in dairy cows on eight farms in the lower Fraser Valley region of British Columbia, Canada

Variable	n[2]	Odds ratio (95% CI)	*P*-value
Average daily standing time (h/d)			
d -14 to d -2	262	1.16 (0.87-1.55)	0.32
d -1 to d 1	253	1.18 (0.94-1.49)	0.21
d 2 to d 14	256	1.40 (1.07-1.86)	0.01
Average daily longest standing bout (h/d)			
d -14 to d -2	262	1.08 (0.78-1.44)	0.60
d -1 to d 1	253	1.07 (0.78-1.43)	0.68
d 2 to d 14	256	1.55 (1.14-2.15)	0.006
Change in average daily standing time[3]	250	1.22 (0.95-1.57)	0.11
Change in average daily longest standing bout[3]	250	1.53 (1.07-2.21)	0.02

[1]Farm included as random intercept.
[2]Number of animals differed between periods because of missing standing data.
[3]Positive value = standing behaviour increased from the prepartum (d -14 to -2) to the postpartum (d 2 to 14) period.

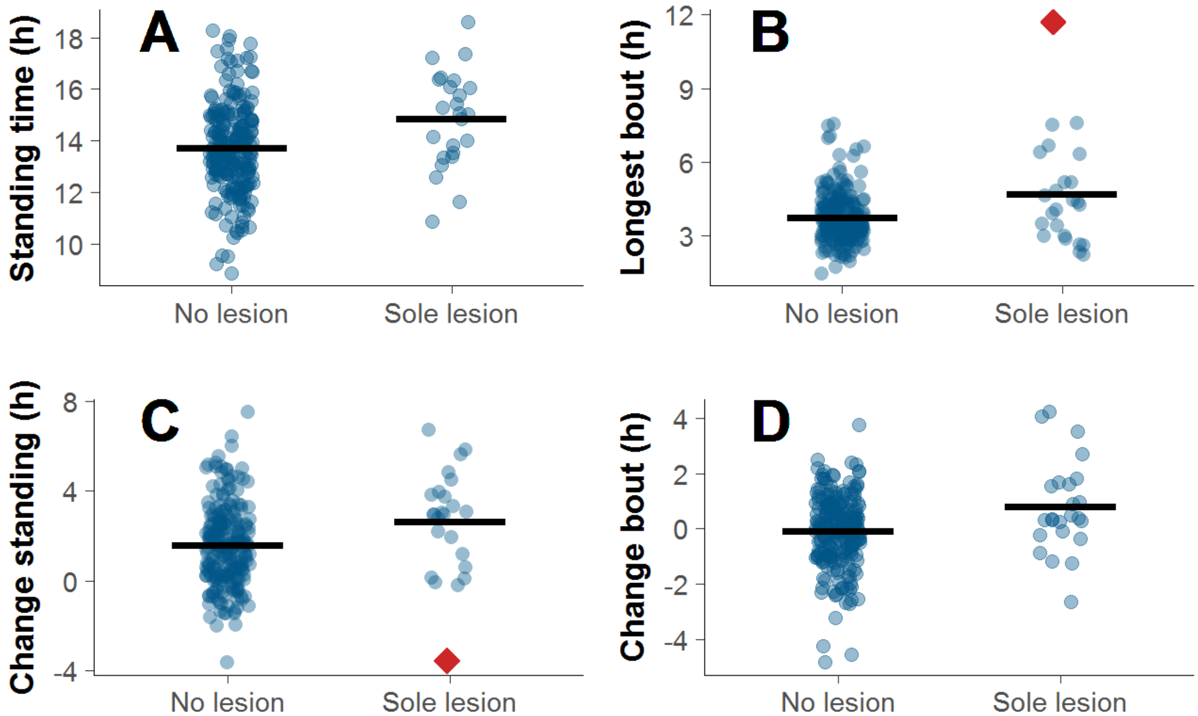

Figure 3.5 Standing behaviour in cows with no or sole lesions at trimming
The animals were trimmed 11±2 wk postpartum. Due to malfunctioning data loggers, 256 cows had recorded standing data postpartum, while 250 cows had data both pre- and postpartum. (A) Average daily standing time postpartum (d 2 to d 14); (B) average daily longest standing bout postpartum (d 2 to d 14); (C) change in average daily standing from before (d -14 to d -2), to after (d 2 to d 14) calving; and (D) change in average daily longest standing bout from before, to after calving. Group means are illustrated with crossbars. Red diamonds illustrate the 2 most influential data points in mixed-effects logistic regression models with farm as random effect, used to evaluate the relationship between standing behaviours and sole lesions; removal of these data points changed model output[1].

[1]Removal of the influential data point in graph B reduced the effect size and increased the standard error for average longest standing bout, which changed the p-value from $P = 0.006$ to $P = 0.052$. As the findings for this animal conformed to the hypothesised higher risk of sole lesions for animals with extreme standing behaviours, the cow was retained in the final model. Removal of the influential data point in graph C increased the effect size for change in average daily standing time, which changed the p-value from $P = 0.11$ to $P = 0.02$. This animal had the third highest average standing time before calving, which decreased to below the median value postpartum. As the cow remained sound during the period standing behaviour was recorded, we found no reason that could justify removing the animal from the final models.

The effects of covariates differed marginally between models (Table 3.5). Parity and milk yield at DIM 50-100 were never associated with sole lesions, but losing ≥1.0 BCS score substantially increased the risk of developing sole lesions.

Table 3.5 Model covariates
Covariates included in multiple mixed-effects logistic regression models[1] used to evaluate the effect of standing behaviours in late gestation and early lactation on the probability of sole lesions at 11±2 wk postpartum in dairy cows on eight farms in the lower Fraser Valley region of British Columbia, Canada

	Odds ratio (95% CI)			
Covariate	Before[2]	Around[2]	After[2]	Change[2]
Standing time models				
BCS at calving				
≥3.25	Referent			
<3.25	1.8 (0.5-6.0)	2.3 (0.6-8.0)	1.9 (0.6-6.2)	2.0 (0.6-6.5)
BCS loss				
≤0.5	Referent			
≥1.0	6.8 (1.7-47.4)	6.7 (1.6-48.2)	7.1 (1.7-48.8)	7.8 (1.9-54.4)
Milk yield (kg)	1.1 (1.0-1.1)	1.0 (1.0-1.1)	1.0 (1.0-1.1)	1.0 (1.0-1.1)
Parity				
1	Referent			
2+	0.5 (0.1-1.8)	0.6 (0.2-2.4)	0.7 (0.2-2.5)	0.6 (0.2-2.2)
DIM at trimming	1.1 (1.0-1.1)	1.0 (1.0-1.1)	1.0 (1.0-1.1)	1.0 (1.0-1.1)
Standing bout models				
BCS at calving				
≥3.25	Referent			
<3.25	1.8 (0.5-6.1)	2.2 (0.6-7.7)	2.2 (0.6-7.0)	2.3 (0.7-7.4)
BCS loss				
≤0.5	Referent			
≥1.0	7.0 (1.7-48.9)	6.5 (1.5-46.0)	6.6 (1.6-46.0)	8.0 (1.9-56.8)
Milk yield (kg)	1.1 (1.0-1.1)	1.0 (1.0-1.1)	1.0 (1.0-1.1)	1.0 (1.0-1.1)
Parity				
1	Referent			
2+	0.5 (0.1-1.7)	0.5 (0.1-2.1)	0.4 (0.1-1.6)	0.5 (0.1-1.8)
DIM at trimming	1.0 (1.0-1.1)	1.0 (1.0-1.1)	1.0 (1.0-1.1)	1.0 (1.0-1.0)

[1]Farm included as random intercept
[2]Period definitions are in relation to calving; before = d -14 to d -2 (n = 262); around = d -1 to d 1 (n = 253); after = d 2 to d 14 (n = 256); change = change in standing behaviour from before, to after calving (n = 250).

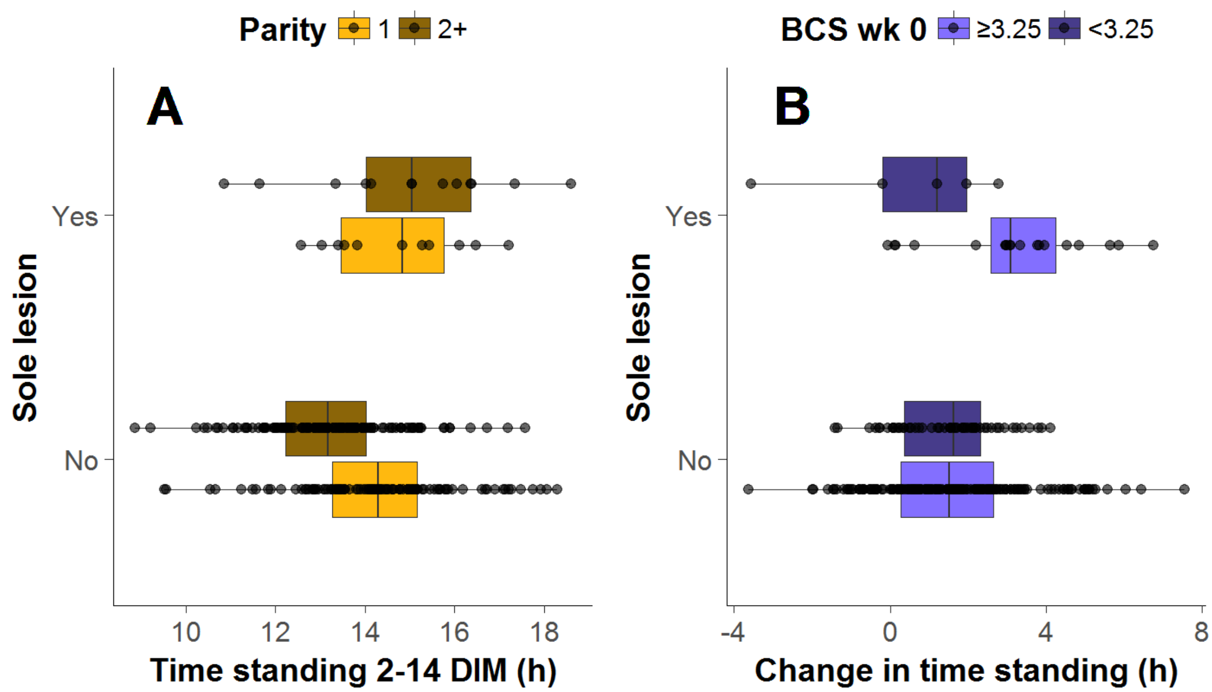

Figure 3.6 Significant interactions
Graphical presentation of significant interactions tested in separate mixed-effects logistic regression models analysing the relationship between standing behaviour and sole lesions. The full models did not converge when the interactions were included. Standing behaviour was summarised/cow into three periods: before (d -14 to d -2), around (d -1 to d 1), and after (d 2 to d 14) calving. (A) Interaction between parity and average daily standing time after calving; (B) interaction between BCS at calving and change in average daily standing time from before to after calving.

3.4 Discussion

The current study is the first large observational study to evaluate the relationship between standing behaviour during the transition period and the development of sole horn lesions on commercial farms. It is also the first study to evaluate the effects of the longest standing bout, instead of only average bout duration.

The relationship between standing behaviour and sole lesions depended on period. We found no association before and around calving, but after calving average daily standing time and average daily longest bout were positively related to sole lesions. The association between standing time and sole lesions may be stronger for multiparous cows, but the interaction term could not be tested in the full model. Animals that increased the duration of the daily longest standing bout from pre- to postpartum had an increased risk of developing sole lesions, which may indicate that a sudden increase in the duration of sustained mechanical load during the transition period is particularly damaging. Newsome et al. (2017a) found that animals with little soft tissue between the sole and the pedal bone were more likely to develop claw horn lesions, and that the sole soft tissue was the thinnest the first week postpartum. Their findings suggest that prolonged weight bearing immediately following calving may be particularly detrimental to claw health, potentially explaining why only postpartum standing behaviour was associated with sole lesions in our study.

When constructing our models statistical indications of collinearity between average standing time and average longest standing bout was present, suggesting that both behaviours were associated with sole lesions through the same underlying mechanism in our study. Animals with high average longest standing bout also had high average standing time within the same period. This finding suggests that it was difficult for cows to compensate for lost lying time due to long standing bouts. Cows that increased the duration of average longest standing bout after calving also increased their average daily standing time, further substantiating the relationship between long standing bouts and standing time.

Parlour-milked cows spend a large part of the day outside of their home-pen during milking, with milking times ranging between 0.7 and 8.0 h/d in a recent Canadian study on 111 farms (Charlton et al., 2014). As dairy cows enter the parlour in a non-random order (Cook and Nordlund, 2009), some animals consistently have a longer return time to the home-pen. This variability in return time is one possible explanation for why the postpartum duration of daily longest standing bout consistently differed between the cows in our study (illustrated by the strong correlation between the longest standing bout during the period and average daily longest standing bout). Indirect evidence for negative effects of long milking duration comes from a cross-sectional study that reported a positive association between time spent away milking and pen prevalence of lameness (53 pens on 50 farms; Espejo and Endres, 2007). Based on the positive relationship between average daily longest standing bout and sole lesions in our study, we propose that animals with long return times from milking are more likely to develop sole lesions, and suggest that further work should evaluate this hypothesis.

The frequent locomotion assessment in our study revealed distinct patterns for the animals that become lame after calving, depending on what type of lesion that was recorded at trimming. Compared to cows with no or claw horn lesions, animals with infectious lesions on average became lame later in lactation. The majority of lame animals without claw lesions at trimming (n = 61) had impaired gait for ≥6 wk before leaving the study, making it unlikely that they were misclassified as lame. Claw assessment was performed by professional trimmers (researchers were not present due to time constraints), and lesions were registered in accordance to a field guide used among certified trimmers in Western Canada. Mild haemorrhages were not registered, so it is likely that some of the lame animals without trim findings had mild claw horn lesions. Alternatively, some of these animals may have been lame due to painful conditions higher up in the leg. Animals with claw horn lesions were most likely to become severely lame after calving, highlighting how large impact this type of claw lesion has on the welfare of dairy cattle.

As multiple studies have shown that claw horn lesions often reoccur (Hirst et al., 2002; Manchado et al., 2011; Foditsch et al., 2016), previous claw horn lesions could potentially have influenced the association between standing behaviour and new sole lesions. For this reason, our study was designed to reduce the risk of including animals with impaired claw health. As a result, a high proportion (44%) of the enrolled animals were first parity animals; interpretation of our result should thus be done in the light of the study's target population. In contrast to earlier research (Amory et al., 2008; Barker et al., 2009; Bicalho et al., 2009), we did not find any association between parity and sole lesion risk, likely because animals with a known history of severe claw horn lesions were not enrolled in the study.

Although both parity groups were equally likely to develop sole lesions, multiparous animals generally had more severe lesions. This finding has been reported also in other studies (Manske et al., 2002a; Sanders et al., 2009), and could partially explain the lower incidence of lameness in primiparous cows in our study. Although there is substantial overlap, sole haemorrhages generally occur earlier than sole ulcers after calving (Newsome et al., 2017b). However, the disparity in lesion severity between parity groups could not explained by differences in trim date relative to calving in the current study. It is possible that the more severe lesions found in multiparous animals could be a result of osteophyte formation on the pedal bone. The protruding bone formations likely increase the focal pressure on the corium during weight load (Newsome et al., 2016). As the size of the osteophytes generally increases with age (Tsuka et al., 2012; Newsome et al., 2016), prolonged standing could be particularly detrimental to the claw health of multiparous animals. Conversely, as most animals with milder horn lesions were primiparous, the reason that 70% remained sound until trimming could be that chronic changes in the deeper structures of the claw had not yet developed in these animals. Although animals with sole haemorrhages were less likely to be lame, it has been suggested that inflammation caused by trauma may induce bone formation on the pedal bone, thereby increasing the risk of recurrent and more severe lesions (Newsome et al., 2016). This claim is substantiated by the findings of Randall et al. (2016), who reported that primiparous cows with severe sole haemorrhages and small sole ulcers 2 to 4 months postpartum (the animals were grouped together for analysis, 89% had haemorrhages) had a higher risk of lameness in future lactations.

We found little evidence of a relationship between BCS at calving and sole lesions; likely because only a few of the animals (3.4% of all cows) were thin (BCS <3.0) at that time point. Hoedemaker et al. (2009) reported an increased risk of lameness for animals with BCS <3.0 at calving, while Green et al. (2014) found that BCS <2.5 in lactating cows was associated with claw horn lesion related lameness 0 to 2, and 2 to 4 months later. One suggested theory for the causal relationship between low BCS and horn lesions is that fat mobilization reduces the size of the digital cushion. This is believed to decrease the force dissipating capacity of the cushion (Bicalho et al., 2009), making the claws more susceptible to mechanical damage. While Newsome et al. (2017a) generally found a positive correlation between the thickness of subcutaneous fat and the distance between the sole horn and the pedal bone (a larger distance indicates a larger digital cushion), this relationship was not present the first week after calving. This finding suggests that factors other than BCS may be more important for the functionality of the digital cushion directly after calving; one potential factor is the increase in laxity of the suspensory apparatus that occurs during this period (Tarlton et al., 2002; Knott et al., 2007).

Although multiple studies have shown that high yielding animals are more likely to become lame (Barkema et al., 1994; Green et al., 2002) and develop claw horn lesions (Amory et al., 2008), we found no association between milk production and sole lesions. It is possible that this unexpected finding was due to decreased milk production in cows that became lame after calving (animals with sole lesion were more likely to become lame). Despite a reduction in milk production due to lameness, high-yielding animals may still produce more than farm average (reviewed by Huxley, 2013). As our primary aim was to obtain unbiased estimates for standing time and longest standing bout, milk yield was retained in the models. Lameness was not included as it was considered an intervening variable for the two standing behaviours.

Recently, Newsome et al. (2017a) suggested that the relationship between BCS loss and claw horn lesions could be confounded by genotypes associated with high milk yield. The authors argued that the more pronounced insulin resistance seen in high producing cows could affect the integrity of the suspensory apparatus through hyperinsulinemia, while higher milk production would result in larger mobilization of body reserves. In our analyses we found a decrease of ≥1 BCS in early lactation to be associated with sole lesions, while simultaneously controlling for milk yield. To further explore the possibility of confounding we removed milk yield from the final models, which resulted in minor changes in the estimates for BCS loss (5.5 – 8.7% increase in slope). As such, there was little statistical support for a complete confounding effect of milk yield in our analyses. For reasons discussed earlier, our milk yield measure may have been an imperfect proxy for high-yielding genotype; additional research should further evaluate this hypothesis.

In comparison with previous estimates of sole lesions (sole haemorrhages: 55 – 68%; Capion et al., 2009; Randall et al., 2016; sole ulcers: 2%; Randall et al., 2016) in primiparous cows (an animal group with lower risk of previous severe claw horn lesions), few of the animals in our study had sole lesions at trimming (sole haemorrhage = 4.3%; sole ulcer = 3.7%). Randall et al. (2016) reported that most of the animals with registered sole lesions in their study had low severity sole haemorrhages, so it is likely that only moderate to severe sole haemorrhages were registered by the trimmers employed in our study. This difference in classification may have made it more difficult to detect a relationship between standing behaviour and sole lesions in the current study. The prevalence of sole ulcers in our study was lower than what was reported in a cross-sectional study performing whole herd lesion assessment (8.3%; Manske et al., 2002b), suggesting that we succeeded in removing some cows with previous CHDL. However, the sole ulcer prevalence in our study was almost twice as high as reported by Randall et al. (2016) for first-calving animals, suggesting that some animals with previous claw problems still remained in our data set.

The enrolled farms were reasonable representative for free-stall herds in BC, Canada (see von Keyserlingk et al., 2012 for comparison). However, the low number of animals with sole lesions made the study underpowered, and some of the interactions suggested in our causal diagram (parity x BCS loss, and parity x BCS at calving) could not be tested due to insufficient data. It is questionable if significant (or non-significant) results for interactions tested in the separate models are robust, considering that some cell counts were very low. Many risk factors for sole lesions are interrelated, and could potentially influence the effect of other predictors. Currently our understanding of how different risk factors interact is incomplete; additional research is needed to fully understand the etiopathogenesis of sole lesions.

3.5 Conclusions

This study evaluated how standing behaviour during the transition period relate to sole lesions in peak lactation. Increased time spent standing, and long standing bouts postpartum were associated with higher odds of severe sole lesions in peak lactation. Animals with claw horn lesions at peak lactation were more likely to become severely lame after calving compared to animals with no recorded claw lesions.

Chapter 4: The effects of the social environment on behaviour and claw health in primiparous cows

4.1 Introduction

Regrouping disrupts behaviour, and may increase competitive behaviour and standing time (von Keyserlingk et al., 2008). These behavioural changes are more pronounced if the regrouping involves more than one stressor, such as simultaneous change of environment and mixing with unknown social partners (Schirmann et al., 2011). Prolonged standing time has been reported when first-calving animals were mixed with older cows for the first time, with focal animals spending on average 83-90% of the measured time standing during the first 8-33 h after regrouping (O'Connell et al., 2008; Boyle et al., 2012; Wagner et al., 2012).

Long standing times, and long time spent standing with only the front feet in the stall (perching), have been related to increased risk of CHDL (Galindo and Broom, 2000; Proudfoot et al., 2010), perhaps because prolonged standing increases the duration of sustained mechanical load on the claws (Ossent and Lischer, 1998). Social competition may increase the risk of CHDL through the same mechanism (Newsome et al., 2016). It is possible that the dramatic increase in the number and severity of claw horn disruption lesions (**CHDL**; sole haemorrhages, sole ulcers, and white line lesions) commonly observed in primiparous cows after the onset of lactation (Whay et al., 1997; Webster, 2002) in part can be explained by a sudden increase in mechanical strain on the claws after mixing with older cows.

The laxity of the suspensory apparatus in the claw increases around calving (Tarlton et al., 2002), which is believed to aggravate the effect of mechanical strain on the claws and increase the risk of CHDL. This argument is supported by studies showing that cows diagnosed with severe sole lesions in mid-lactation spent more time standing around calving compared to cows that maintained good claw health (Chapinal et al., 2009b; Proudfoot et al., 2010).

The negative behavioural effects of regrouping can be reduced if cows are housed in a known environment (Schirmann et al., 2011), or regrouped to a pen with low stocking density (Talebi et al., 2014). Hence, by altering the way first-calving animals are managed when they transition into lactation, it may be possible to influence to what extent standing behaviour is affected by regrouping, thereby reducing the risk of CHDL after calving. Although the effect of overstocking on behaviour is well researched (e.g. Olofsson, 1999; Hill et al., 2009; Krawczel et al., 2012), few studies have focused on the effect of understocking (but see Telezhenko et al., 2012; Talebi et al., 2014; Winckler et al., 2015); we are not aware of any study that have evaluated the effects of regrouping first-calving animals to low-stocked pens after calving.

Our primary objective was to evaluate how the post-calving social environment affected the behaviour and claw health of newly calved heifers. We predicted that animals regrouped to a low-stocked pen with peers of similar age would 1) spend less time standing and perching after regrouping, and 2) have less severe CHDL in peak lactation, than would heifers mixed with multiparous cows after calving. Our secondary objectives were to explore how social competition after regrouping was related to the development of CHDL, and to describe how the social environment influenced the time-budget of primiparous cows during the onset of lactation.

4.2 Material and methods

The study was performed at the University of British Columbia's Dairy Education and Research Centre (Agassiz, British Columbia, Canada) from May 2015 to Feb 2016, under UBC

Animal Care Committee protocol number A14-0040. During the study period the average ambient temperature was 18.8° C (range 1.6-35.7° C).

4.2.1 Sample size

A power analysis was performed to detect differences in standing time between treatments. Based on Proudfoot et al. (2010), who reported 2 h/d longer standing time in transition cows that developed severe sole lesions in peak lactation compared to animals that maintained good claw health, a power calculation to compare two independent means were performed ($\alpha = 0.05$; $1\text{-}\beta = 0.8$), assuming that the standard deviation of standing time was 2 h (Endres and Barberg, 2007). The analysis resulted in an estimate of 16 animals required in each treatment to detect an average difference in standing time of at least 2 h.

4.2.2 Study animals

All heifers in late gestation that had not previously been housed together with multiparous animals were eligible for enrolment. Claw health was evaluated wk -6 in relation to expected calving date, and corrective trimming was performed if the claws were overgrown. Animals with abnormal claw shape (cork-screw claws) or severe CHDL (sole ulcer, white line disease and toe ulcer) were not further considered for enrolment.

A total of 42 Holstein heifers 672±31 d (mean±SD) of age were enrolled in the study 41±4 d before expected calving date on an on-going basis. Enrolled heifers were housed and managed together until calving; animals that calved in late summer and autumn had been kept on pasture before being enrolled in the study. Approximately 3 wk before expected calving, the heifers were moved to the building where the study was conducted; they were then housed separate from multiparous cows in a dynamic pen with stocking density maintained at one animal/lying stall until calving (see Figure 4.1 for pen-layout).

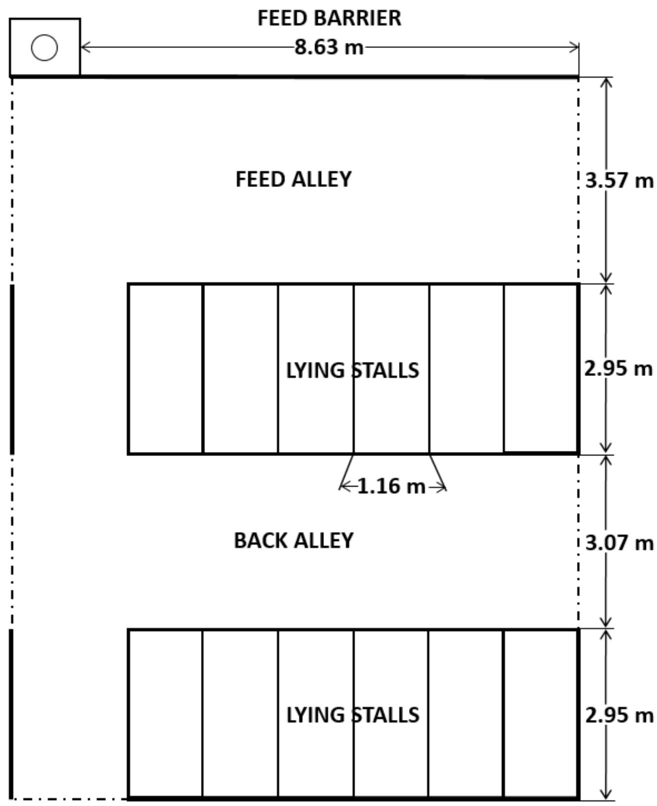

Figure 4.1 Schematic of one of the pens used in the study
Each pen (9.8 x 12.5 m) contained 12 free-stalls bedded with 0.4 m of sand in two rows. The distance between the brisket board and the rear curb was 2.03 m, and the height of the rear curb was 0.18 m. The distance between the stall surface and the neck rail was 1.20 m, while the distance between the neck rail and the back curb was 2.02 m. The feed barrier contained 13 head-locks with 0.61 m linear space/head-lock, and a focal water source (circle surrounded by rectangle). Dot-dashed lines illustrate pen gates.

The heifers were moved to an open pack maternity pen deep-bedded with straw when signs of imminent calving were observed (i.e. restlessness, udder enlargement, relaxation of the sacrosciatic ligament). Calving time for each animal was determined from video recordings, and the heifers were alternately assigned to two different treatments based on the order they calved.

Thirteen heifers calved in the prepartum pen; these animals were directly transferred from the prepartum pen to their postpartum treatment pens and balanced across the two treatments. Treatments were also balanced based on body weight (kg) measured 2 wk before calving (612±40 vs. 604±42 kg), the recorded body weight was an average of two measurements taken on consecutive days.

4.2.3 Experimental design

Twenty-two heifers were housed in a dynamic, low stocked treatment pen (**LS**; 12 stalls and 13 head-locks/≤4 animals) with familiar primiparous cows, directly adjacent to the prepartum pen. The remaining animals (n = 20) were mixed with pregnant multiparous cows in one of two dynamic control pens stocked according to recommended best practice (**CON**; 12 stalls and 13 head-locks/12 animals). To minimise social contact between familiar CON animals, physical contact between the control pens was prevented by partitions. Only one study animal was allowed to enter each postpartum study pen per day, and no more than four experimental heifers were present in the study pens at any point (see Figure 4.2 on page 99). The use of the study pens was reversed after every 12th calving, to eliminate potential side bias. The animals were kept in their allotted pens until approximately 3 wk postpartum (LS: 20.0±1.2 d; CON: 20.3±1.3 d), after which they were moved to a dynamic control pen as described previously for the CON animals. The study animals were kept in the new control pen for approximately 5 d (LS: 4.8±1.0 d; CON: 4.7±1.1 d; Figure 4.2), before being moved to the main lactating herd. Care was taken so that the main herd pens contained the same number of animals from both treatments, and that the ratio of primi- and multiparous cows was close to the average of the farm (30 – 35% primiparous cows).

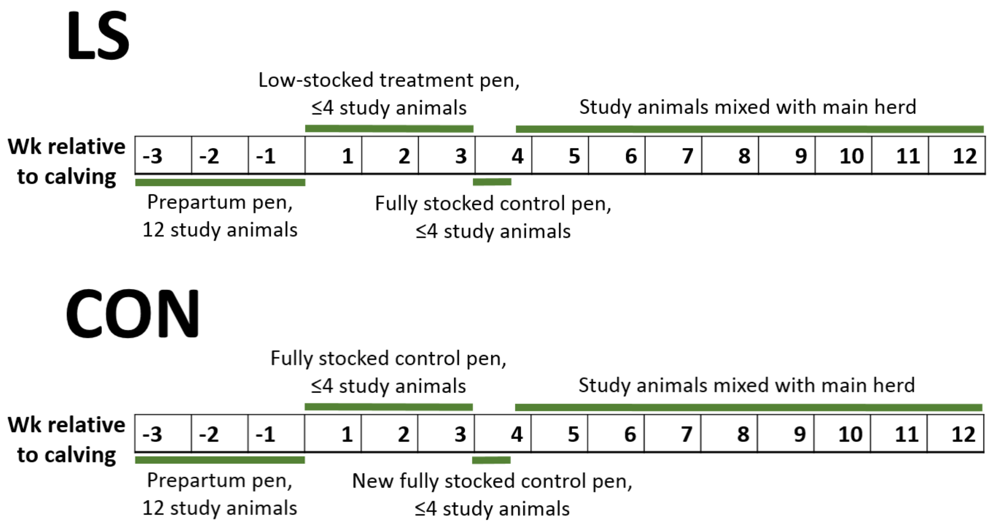

Figure 4.2 Study treatments
(LS) Primiparous animals kept at ≤33% stall-stocking density with familiar animals of the same age after calving; (CON) primiparous animals kept at 100% stall-stocking density and mixed with multiparous cows after calving.

4.2.4 Housing and management

From 3 wk before calving until they were moved to the lactating herd the heifers were housed in a naturally ventilated building adjacent to the main barn. The experimental pens each contained 12 sand-bedded free-stalls with Y2K dividers in two rows (see Figure 4.1 on page 97). The pens had vulcanised rubber floor (KURA P, Gummiwerk KRAIBURG Elastik, Tittmoning, Germany), which was cleaned with scrapers 6 times/d. Each pen had a feed barrier with 13 head-locks (Self Locks, Artex Global Barn Solution Providers, Abbotsford, BC, Canada). Water was provided by an electronic watering system (Insentec, Hokofarm Group, Marknesse, the Netherlands) at the feed barrier, which could be accessed by one cow at the time.

After they were moved to the main herd, the study animals were housed in pens with 24 – 36 sand-bedded free-stalls in three rows, and ≥0.6 m of post-and-rail feed barrier/animal (Artex Global Barn Solution Providers, Abbotsford, BC). In these pens, the floor adjacent to the feed-barrier (1.9 m) was covered with vulcanised rubber (Farm Tech, Abbotsford, BC, Canada), while the flooring in the rest of the pen was grooved concrete. Water was freely available from a 1.8 m long self-filling trough located on the elevated cross-over between the feed and the back alley.

For prepartum animals, dry-cow total mixed ration was delivered once daily at approximately 0830 h. Lactating animals were provided fresh total mixed ration twice daily at approximately 0600 and 1530 h. During the first 3 wk postpartum the morning milking occurred between 0730 and 0815 h and evening milking between 1730 and 1815. The animals spent approximately 1 h outside the pen/milking.

4.2.5 Behaviour recording

Behaviours were continuously video recorded from calving until the study animals were moved to the main herd (Geovision 1480 digital recorder, USA Vision Systems, Irvine, CA, US) using two cameras (WV-CW504SP, Panasonic, Osaka, Japan) in each postpartum study pen. One camera was mounted 6 m above the feed alley and the other 5.5 m above the lying stalls. Red lights (100 W) were hung adjacent to the cameras to facilitate video recording at night. The heifers were marked with unique alphanumeric symbols using hair dye to allow for individual identification from video. Total daily time standing (i.e. time spent in upright position, including time feeding and milking) and time spent perching during the first 3 d after calving were measured for all animals using 5-min instantaneous scan sampling.

For a subset of heifers (LS = 12 animals; CON = 12 animals) more detailed behavioural data were collected (see Table 4.1 on page 101 for definitions), as well as the time of day and where in the pen the behaviours were performed (feed alley, back alley or free-stalls). This subset was balanced for calving date and body weight (LS = 607±42 kg; CON = 602±37 kg). Using 5-min scan sampling, the time-budget of these animals was recorded the first 3 d after calving, d 7 postpartum, the last 24 h in the post-calving pen, and for 3 d following regrouping at d 21. For the same subset of animals aggressive interactions (Table 4.1) at the feed-bunk and water bin were scored during the first 3 d in each postpartum study pen. The video was assessed continuously for 90 min following the two daily feedings (DeVries and von Keyserlingk, 2005); if animals were brought up for milking before 90 min had passed, recording continued when the cows returned until a total of 90 min had been scored.

Table 4.1 Ethogram describing recorded behaviours
Video from primiparous cows kept in either a low-stocked pen with familiar conspecifics of the same age (n = 12) or in fully stocked pens mixed with multiparous animals (n = 12) for 3 wk after calving was assessed to evaluate how the time-budget, and amount of aggressive interactions depended on treatment. After 3 wk both treatments were housed in fully stocked pens mixed with older animals.

Behaviour	Definition
Lying[1, 2]	The ventral part of the abdomen is in contact with the ground
Feeding[1, 2]	The head of the cow is completely past the feed barrier and over the feed
Standing or walking[1, 2]	The cow is maintaining an inactive upright position or is walking
Perching[1, 2]	The cow is standing with only the front feet in the free-stall
Four feet standing[1, 2]	The cow is standing with all four feet in the free-stall
Actor event[3]	Butt/push from the focal cow immediately results in complete withdrawal of the other cow's head from under the feed barrier
Reactor event[3]	Butt/push from another cow immediately results in complete withdrawal of the focal cow's head from under the feed barrier

[1]Behaviours measured over 24-h using 5-min instantaneous scan sampling.
[2]The location the behaviour was performed was recorded as 1) the feed alley, 2) the back alley, and 3) the free-stalls.
[3]Behaviours recorded from continuous video for 90-min after feeding.

The video recordings were scored by three jointly trained observers. Respectively, the observers scored 60%, 32% and 8% of the videos, with each observer scoring approximately equal number of videos for both treatments. Inter-observer reliability was evaluated every 5th video, while intra-observer reliability was assessed on two occasions (midway and end of study period) after the initial training period. Observer reliability for instantaneous scan sampling was evaluated with unweighted kappa using the irr package (Gamer et al., 2012), and almost perfect agreement was achieved ($\boldsymbol{K} \geq 0.95$; Landis and Koch, 1977). Consistency in scoring aggressive interactions was evaluated with percent agreement (intra-observer ≥88%; inter-observer ≥74%).

4.2.6 Claw lesions

Front and back hooves were examined at wk 6 and wk 12 postpartum to allow for lesions developing around calving to become visible on the sole surface (Livesey et al., 1998). Lesions were scored by the same trained observer that assessed the heifers before enrolment. Before examination the claws were cleaned, and approximately 1 mm of the sole horn was removed. The number, location, and severity of sole haemorrhages, sole ulcers, white line haemorrhages, and white line separation were recorded on a standardised form, as was the presence and severity of heel horn erosion, digital dermatitis, foot rot and interdigital hyperplasia (see Table 4.2 on page 103 for definitions). The size of the claw horn lesions where not recorded, as lesion severity has a stronger association with lameness than lesion size (Whay et al., 1997). The data were summarised by cow and period; a cow was considered affected with a lesion if it was found on at least one claw. If more than one type of lesion was present, the animal was considered affected with all lesion types identified.

Table 4.2 Description of recorded claw lesions
The hooves of primiparous cows were examined at wk -6, 6 and 12 in relation to calving.

Description of claw lesion	Score	Reference
Sole and white line lesion		Leach et al., 1998
Diffuse red or yellow discolouration	1	
Stronger red discolouration	2	
Deep dense red lesion	3	
Port-coloured lesion	4	
Red coloured, raw and possibly bleeding lesion	5	
Exposed corium	6	
Major horn loss with exposed corium	7	
Infected lesion with exposed corium	8	
White line separation		Smilie et al., 1999
Dark striation of white line	1	
Small crevice between claw wall and sole horn	2	
Moderate separation	3	
Complete separation	4	
Heel horn erosion		Smilie et al., 1999
Shallow irregular depressions	1	
Deep irregular depressions	2	
Shallow oblique grooves	3	
Deep oblique grooves with exposed corium	4	
Digital dermatitis		Manske et al., 2002
Red area with erect pili	1	
Moist red area covered with discharge but with intact epidermis	2	
Exudative area with exposed corium, no signs of healing	3	
Brown scab covering exposed corium, epidermis is healing	4	
Brown scab covering healed epidermis	5	

The sole of each hoof was photographed on each claw examination, holding the camera (DMC-FT1, Panasonic, Osaka, Japan) at approximately 20 cm distance from the hoof and perpendicular to the sole surface. The photographs had 4000 x 3000 pixel resolution, with sRGB colour representation. To test repeatability of claw horn lesion scoring, one good quality photograph per claw from each examination was coded and scored for presence of sole and white line lesions with severity ≥3 in a randomised order by the initial observer and an observer blind to treatment (Table 4.3 and Table 4.4).

Table 4.3 Inter-observer agreement for scoring of sole lesions
Coded photographs of the sole were scored for the presence of sole lesions with severity ≥3 by two observers blind to treatment. One observer had previously evaluated claw lesions during claw trimming performed wk -6, wk 6 and wk 12 in relation to calving.

		Observer 1		
		No lesion	Sole lesion	Total
Observer 2	No lesion	377	12	389
	Sole lesion	37	54	91
	Total	37	66	480

Table 4.4 Inter-observer agreement for scoring of white line lesions
Coded photographs of the sole were scored for the presence of white line lesions with severity ≥3 by two observers blind to treatment. One observer had previously evaluated claw lesions during claw trimming performed wk -6, wk 6 and wk 12 in relation to calving.

		Observer 1		
		Sound	White line lesion	Total
Observer 2	No lesion	346	6	352
	White line lesion	63	65	128
	Total	409	71	480

Inter-observer reliability was evaluated using the kappa statistic; as the kappa statistic assumes data independence, separate kappa values were calculated per claw. The analyses indicated moderate to substantial agreement (Landis and Koch, 1977) for the presence of sole lesions ($\boldsymbol{K}$ = 0.43 – 0.67) and white line lesions ($\boldsymbol{K}$ = 0.45 – 0.72). To evaluate the effects prevalence and observer bias on the reliability measures, prevalence index (**PI**) and bias index (**BI**) were calculated (Byrt et al., 1993). For sole lesions PI varied between 0.43 to 0.84 depending on claw, and BI varied between -0.01 to -0.11. These results suggest that it was difficult for the observers to identify sole lesions of severity ≥3 from photographs (Vach, 2005), but that the observers showed little systematic bias in their scoring (Sim and Wright, 2005). The results for white line lesions were similar but indicated a slightly larger bias between the observers (PI = 0.34 to 0.71; BI = -0.11 to -0.15).

Lesion data recorded at the claw examinations were compared against how the initial observer scored the coded photographs, and BI was calculated to evaluate systematic differences in scoring. The results suggest that the initial observer did not differ in how LS (sole lesions: BI = 0.00; white line lesions: BI = 0.01) and CON (sole lesions: BI = 0.04; white line lesions: BI = -0.02) animals were assessed at live scoring. As the appearance of recorded claw lesions was not perfectly reproduced in the photographs, the original data were used when analysing claw lesions.

4.2.7 Data handling

Data handling and analyses were performed in R version 3.4.4 (R Core Team, 2018; RStudio Team, 2016; dplyr, Wickham et al., 2017; tidyr, Wickham and Henry, 2018). Data visualization was performed using the ggplot2 package (Wickham, 2009). Days when the behaviour of the animals was affected for >2.4 h were recorded as missing; common reasons were e.g. calving occurring in a neighbouring pen, and heat in the study animal. Days when malfunctioning video recording prevented video assessment for >2.4 of the time were also recorded as missing, as were post-feeding and post-milking periods when e.g. stall maintenance influenced the behaviour of the animals.

When analysing if total daily time standing and time spent perching differed between treatments after regrouping, time performing the behaviours was averaged over the first 3 d after calving to obtain average daily time standing and perching in h/d for each animal.

When evaluating if the level of social aggression following regrouping differed between treatments, actor and reactor events were first summarised separately by cow and day. As the time spent in the feed alley differed between animals, the number of actor and reactor events/h present in the feed alley was calculated. Post-feeding periods when the focal animal did not enter the feed alley were recorded as missing. Before the analyses, daily rate data were averaged over the first 3 d in the post-calving pen, and in the second postpartum pen, respectively.

For analyses of claw horn lesions in peak lactation the animals were dichotomized as having lesion severity <3 vs. lesion severity ≥ 3, as recommended by Leach et al. (1998), at each claw assessment; sole and white line lesions were analysed separately.

As little is known about the effect of the social environment on the behaviour of free-stall housed dairy heifers, our hypothesis only concerned standing behaviour. For this reason we only report descriptive results for the time-budget of the animals. When recording the 24-h time-budget, the location was also considered when summarising the data. Daily time feeding, time standing or walking in the feed alley, time standing or walking in the back alley, time lying, time perching and time standing with all feet in the stalls were obtained for each animal in the subset. To explore the social environment's effect on the time-budget during high-activity periods the same behaviours were assessed for 90 min after the animals returned from milking (DeVries and von Keyserlingk, 2005). This period was chosen because the post-feeding period was frequently interrupted by milking, particularly for the CON animals. To capture potential differences in behaviours between treatments directly after regrouping, data from the first 3 d in the two postpartum study pens were summarised by animal. To evaluate if potential differences in time-budgets between treatments persisted during the period LS animals were housed in the low-stress social environment, video data from d 7 and d 21 postpartum were also assessed.

4.2.8 Statistical analyses

The individual animal was considered the experimental unit in all analyses. Continuous data were graphically evaluated for normality and homoscedasticity before statistical analyses. Of the 42 enrolled animals, one LS animal was excluded as it calved before cameras had been mounted in the study pens. Another LS animal left the herd before the second claw examination due to low milk production, data collected before censoring were included in the analyses. Video was assessed for a total of 243 cow-days, of which 10 d (4.1%) were recorded as missing due to camera faults (three days), routine management (i.e. adding sand to stalls; three days), calving in a nearby pen (three days), heat in the study animal (one day) and one animal returning late from milking (one day). Nineteen of 288 (6.6%) post-feeding periods, and 32 of 384 (8.3%) post-milking periods were recorded as missing for similar reasons. Missing video was most prevalent on d 21, when 21 animals (LS: 10 animals; CON: 11 animals) had acceptable recordings. One animal lacked claw assessment at wk 6 (LS animal), and one at wk 12 (CON animal), resulting in 20 LS and 20 CON animals being examined at wk 6, and 20 LS and 19 CON animals examined at wk 12.

4.2.8.1 Hypothesis testing

Total daily time standing and time spent perching d 1 to d 3 postpartum, and number of actor and reactor events/h present in the feed alley all had non-normal distribution; two-tailed

Mann-Whitney U tests were used to analyse if the groups differed in these behaviours. Two-tailed Fisher Exact tests were used to investigate if the number of animals with sole and white line lesions of severity ≥3 differed between treatments.

4.2.8.2 Data exploration

Behavioural data from the first 3 d post-calving were used to explore the relationship between aggressive interactions and sole and white line lesions with severity ≥3. The effects of actor event rate, reactor event rate, and rate of social interactions (actor and reactor events combined) were tested in separate logistic regression models fitted with maximum likelihood. Total daily time standing and time spent perching were included as covariates. Pearson's correlation coefficient was calculated for all linearly correlated predictors, and Spearman's rank correlation coefficient for predictors with non-linear relationship. Signs of strong collinearity were not found between the predictors ($-0.42 < r < 0.47$; $n = 24$). The two-way interactions between time standing and rate of actor events, reactor events, and social interactions were tested in the models, but were not retained ($P > 0.05$). Likelihood ratio tests were used to compare subsets of models, to assess if additional terms improved model fit. As we did not have a priori hypothesis for these analyses the results should be interpreted with caution.

4.3 Results

4.3.1 Hypothesis testing

4.3.1.1 Standing behaviour d 1 to 3

Behaviour recordings from 41 animals were used in these analyses. Figure 4.3 on page 109 illustrates the total standing time and time spent perching after the post-calving regrouping.

The median total daily time standing d 1 to d 3 postpartum did not differ between LS [14.6 h/d, interquartile range (IQR): 13.7 – 16.4 h/d] and CON animals (15.4 h/d, IQR: 13.1 – 16.4 h/d; $U = 229$; $n_1 = 21$, $n_2 = 20$; $P = 0.63$). Median time spent perching was also similar in the two treatments (LS: 2.3 h/d, IQR: 1.6 – 3.8 h/d; CON: 2.4 h/d, IQR: 1.3 – 4.1 h/d; $U = 200$; $n_1 = 21$, $n_2 = 20$; $P = 0.80$).

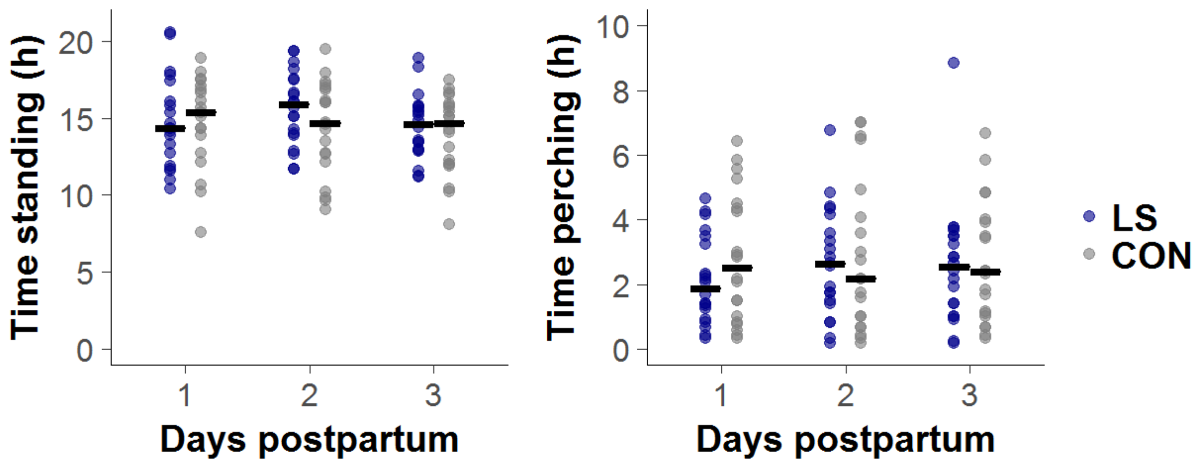

Figure 4.3 Time spent standing and perching
Total time standing and time spent perching d 1 to d 3 after calving. Primiparous cows were kept in either a low-stocked pen with familiar conspecifics of the same age (LS; n = 21) or in fully stocked pens mixed with unknown multiparous animals (CON; n = 20) after calving. The crossbar illustrates the median.

4.3.1.2 Claw lesions

At the prepartum assessment there was no difference between the treatments in the number and severity of recorded claw horn lesions per animal (see Figure 4.4 on page 110), or in the number of animals that had sole (LS: five animals; CON: four animals), or white line lesion of severity >3 (LS: one animal; CON: no animal).

After calving, the number and severity of CHDL per animal increased (Figure 4.4). All but one animal had sole and white line haemorrhages at wk 6, and all did so at wk 12. No animal developed so severe CHDL that the corium was exposed (sole ulcers and white line disease) during the study period. In total 567 sole and 569 white line lesions were recorded postpartum. The majority of the lesions were mild, with 82%, 9.5%, 6.3%, 1.6% and 0.4% of the sole lesions and 70%, 18%, 12%, 0.2% and 0.4% of the white line lesions being severity 1, 2, 3, 4, and 5, respectively.

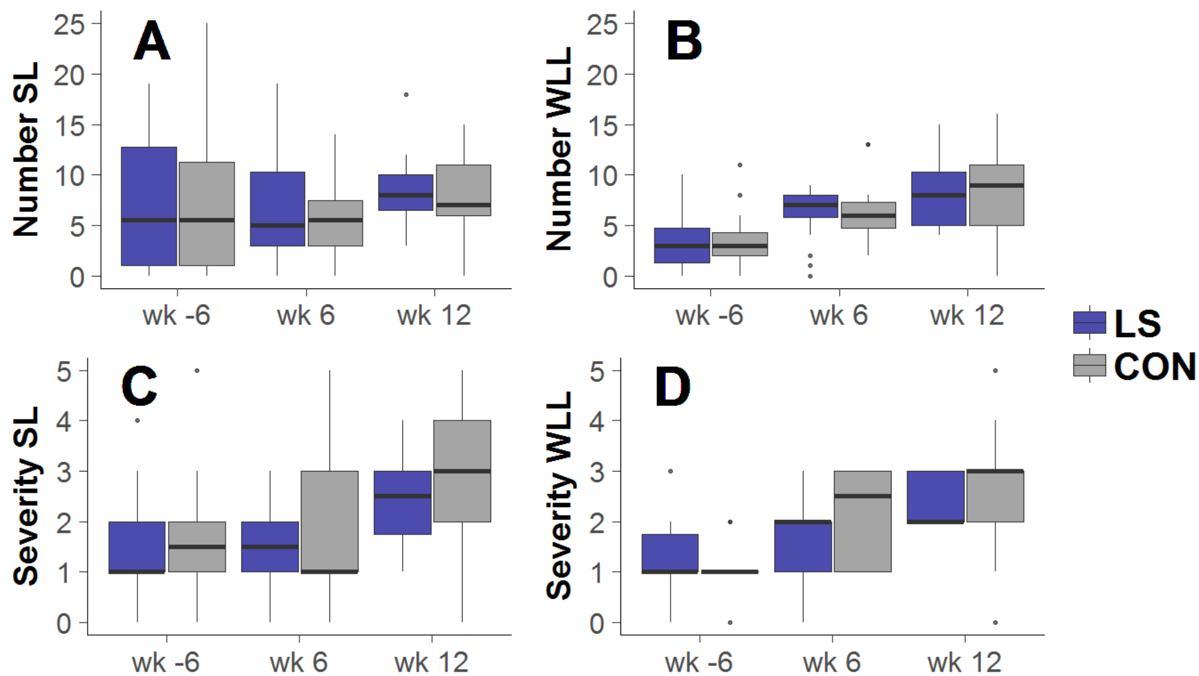

Figure 4.4 Number and severity of CHDL
Descriptive statistics for recorded claw lesions; the number of (A) sole lesions and (B) white line lesions, and the severity of the most serious (C) sole lesion and (D) white line lesion/animal. The hooves were examined at wk -6 (pre-study assessment), and at wk 6 and wk 12 in relation to calving. Primiparous cows were kept in either a low-stocked pen with familiar conspecifics of the same age (LS) or in fully stocked pens mixed with multiparous animals (CON) after calving.

Sole lesions with severity ≥3 were found in four of 20 LS animals and six of 20 CON animals at wk 6 [P = 0.72; odds ratio (**OR**) = 1.7; 95% CI 0.32 – 9.94; Fisher Exact test], and in 10 of 20 LS animals and 12 of 19 CON animals at wk 12 (P = 0.52; OR = 1.7; 95% CI 0.40 – 7.5; Fisher Exact test). Four of 20 LS animals and 10 of 20 CON animals had white line lesions with severity ≥3 at wk 6 (P = 0.10; OR = 3.9; 95% CI 0.83 – 21.7; Fisher Exact test), while at wk 12 nine of 20 LS animals and 12 of 19 CON animals (P = 0.34; OR = 2.1; 95% CI 0.49 – 9.17; Fisher Exact test) were affected. Three CON animals had sole haemorrhages of severity ≥4 at wk 6, and one LS and six CON animals at wk 12. No animal had white line lesion of severity ≥4 at wk 6, while two CON animals were affected at wk 12.

Except for mild white line separations, the prevalence of other claw lesions was low. At wk 6 postpartum 7.3% of the animals had digital dermatitis, 4.9% had heel horn erosion and 34% had mild white line separation, and at wk 12 15% had digital dermatitis, 7.7% heel horn erosion and 26% mild white line separation.

4.3.1.3 Social environment

Treatments did not differ in the median rate of actor events during the first 3 d after regrouping to the post-calving pen (LS: 0.1 actor events/h vs. CON: 0.4 actor events/h; U = 72; n_1 = 12, n_2 = 12; P = 1), or after regrouping to the second postpartum pen (LS: 1.0 actor events/h vs. CON: 0.8 actor events/h; U = 84; n_1 = 12, n_2 = 12; P = 0.51). In the post-calving pen LS animals had a lower median rate of reactor events (2.1 reactor events/h) compared to the CON animals (6.5 reactor events/h; U = 13; n_1 = 12, n_2 = 12; P <0.001; see Table 4.5 on page 113), while we did not observe a difference in the second postpartum pen (LS: 4.3 reactor events/h vs. CON: 4.6 reactor events/h; U = 70; n_1 = 12, n_2 = 12; P = 0.93; see Figure 4.5 on page 112).

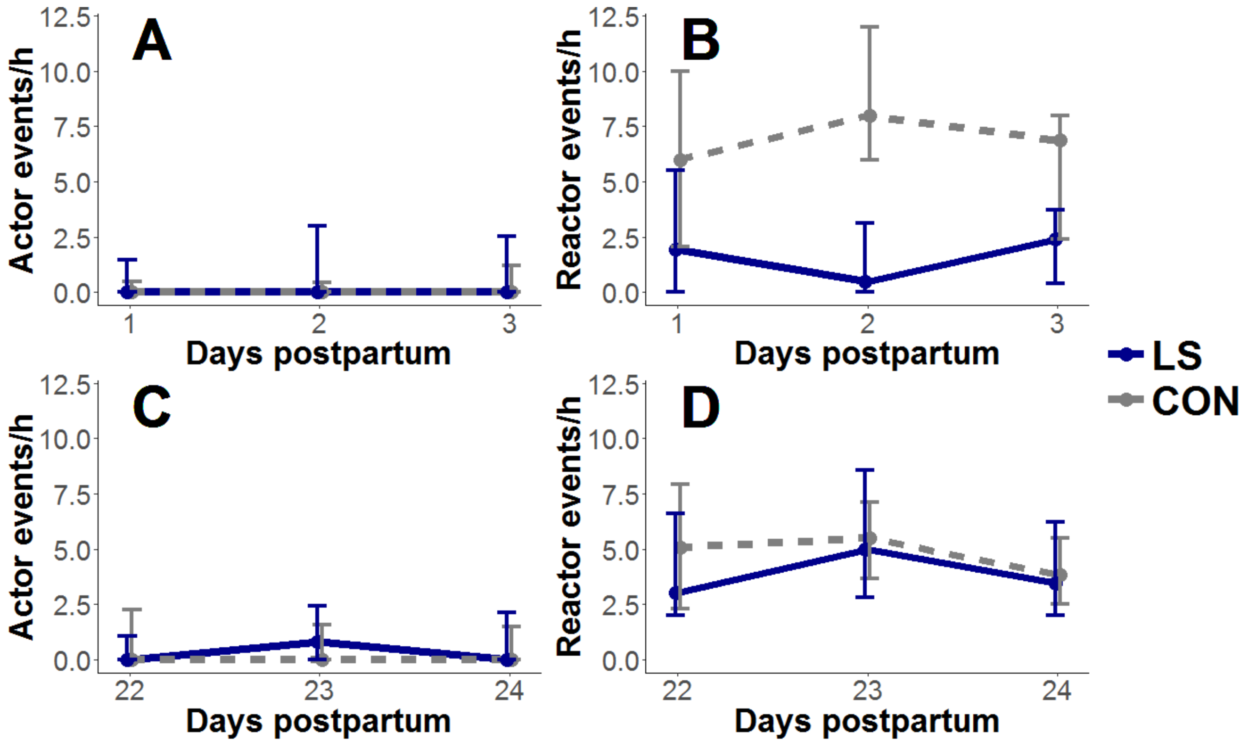

Figure 4.5. Aggressive interactions at the feed-bunk during 90 min following feed delivery Primiparous cows were kept in either a low-stocked pen with familiar conspecifics of the same age (LS; n = 12) or in fully stocked pens mixed with unknown multiparous animals (CON; n = 12) after calving. After 3 wk both treatments were mixed with unknown multiparous animals. The rate of aggressive interactions was defined as number of actor events (focal animal displaces another cow) and reactor events (focal animal is displaced) per h the focal animal spent in the feed alley. (A) The actor event rate was low in both treatments the first 3 d in the post-calving pen; (B) during the same period the rate of reactor event was higher for CON animals. When both treatments were mixed with older animals there was no difference in (C) actor event rate, or (D) reactor event rate. Lines and error bars represent median and interquartile range.

Table 4.5 Descriptive statistics for aggressive behaviours at the feed-bunk
Actor and reactor events were recorded during 90 min after feed delivery. Primiparous cows were kept in either a low-stocked pen with familiar conspecifics of the same age (LS) or in fully stocked pens mixed with multiparous animals (CON) after calving. After 3 wk both treatments were housed in fully stocked pens mixed with older animals.

Day[1]	Trt	n[2]	Time in feed alley (h)[3]	Actor events[4]	Actor events per hour[5]	Reactor events[6]	Reactor events per hour[7]
1	LS	12	0.59±0.25	0 (0-1.00)	0.00 (0-1.46)	1 (0.00-3.00)	1.95 (0.00-5.50)
	CON	12	0.37±0.29	0 (0-0.00)	0.00 (0-0.50)	2 (0.00-4.25)	6.00 (2.06-10.0)
2	LS	11	0.49±0.25	0 (0-1.00)	0.00 (0-3.00)	0 (0.00-1.00)	0.50 (0.00-3.11)
	CON	12	0.45±0.28	0 (0-0.00)	0.00 (0-0.43)	4 (1.50-5.50)	8.00 (6.00-12.0)
3	LS	11	0.55±0.34	0 (0-1.00)	0.00 (0-2.53)	1 (0.00-2.00)	2.40 (0.40-3.71)
	CON	12	0.52±0.29	0 (0-1.00)	0.00 (0-1.20)	2 (0.00-6.00)	6.86 (2.40-8.00)
22[8]	LS	12	0.86±0.32	0 (0-1.00)	0.00 (0-1.05)	3 (1.50-5.50)	3.00 (2.00-6.60)
	CON	11	0.89±0.35	0 (0-2.75)	0.00 (0-2.25)	4 (2.00-5.75)	5.07 (2.29-7.94)
23	LS	10	0.87±0.42	1 (0-2.25)	0.82 (0-2.44)	3 (2.00-9.00)	5.00 (2.80-8.57)
	CON	11	0.98±0.38	0 (0-1.75)	0.00 (0-1.58)	5 (3.25-5.75)	5.50 (3.65-7.11)
24	LS	12	0.93±0.34	0 (0-2.00)	0.00 (0-2.14)	3 (1.00-7.00)	3.43 (2.00-6.20)
	CON	12	0.90±0.35	0 (0-1.25)	0.00 (0-1.50)	3 (1.75-5.00)	3.85 (2.53-5.50)

[1]Day in relation to calving.
[2]The number of animals differed between days because of external factors that interfered with video assessment.
[3]Mean±SD.
[4]Number of times a focal animal displaces another cow; median (IQR).
[5]Number of actor events/h the animal was present in the feed alley; median (IQR).
[6]Number of times a focal animal is displaced by another cow; median (IQR).
[7]Number of actor events/h the animal was present in the feed alley; median (IQR).
[8]First day in the second postpartum pen; at this time all animals were mixed with multiparous cows.

Only 50% of the LS animals and 75% of the CON animals performed actor behaviours during the first 3 d in the post-calving pen. Of the study animals that performed actor behaviours, LS animals performed more actor events/h than did CON animals (LS: 2.7 actor events/h vs. CON: 1.0 actor events/h; $U = 45$; $n_1 = 6$, $n_2 = 9$; $P = 0.04$). After regrouping to the second postpartum pen, most animals performed actor behaviours (LS: 12 of 12 animals; CON: 10 of 12 animals). All but one focal cow performed reactor behaviours in the post-calving pen, and all did so in the second postpartum pen.

4.3.2 Data exploration

4.3.2.1 Social competition and CHDL

We found no relationship between the presence of sole and white line haemorrhages of severity ≥3 at wk 6 and the rate of actor events (sole lesions: OR = 1.21; 95% CI = 0.57 – 2.30; $P = 0.57$; white line lesions: OR = 0.78; 95% CI = 0.29 – 1.49; $P = 0.50$), reactor events (sole lesions: OR = 0.95; 95% CI = 0.68 – 1.30; $P = 0.75$; white line lesions: OR = 1.28; 95% CI = 0.90 – 1.95; $P = 0.17$) and social interactions (sole lesions: OR = 0.99; 95% CI = 0.73 – 1.34; $P = 0.96$; white line lesions: OR = 1.19; 95% CI = 0.84 – 1.81; $P = 0.32$). Likewise, the rate of reactor events (sole lesions: OR = 1.04; 95% CI = 0.78 – 1.39; $P = 0.79$; white line lesions: OR = 1.08; 95% CI = 0.80 – 1.53; $P = 0.61$) and social interactions (sole lesions: OR = 0.90; 95% CI = 0.67 – 1.17; $P = 0.42$; white line lesions: OR = 0.98; 95% CI = 0.73 – 1.32; $P = 0.89$) were not associated with sole and white line lesions at wk 12. However, we found a negative relationship between the rate of actor events and sole haemorrhages of severity ≥3 at wk 12 postpartum (OR = 0.21; 95% CI = 0.03 – 0.71; $P = 0.004$). A negative relationship between actor event rate and white line haemorrhages was also observed, but this association were less pronounced (OR = 0.54; 95% CI = 0.15 – 1.18; P = 0.15).

4.3.2.2 Time-budgets

Daily feeding times were similar in the two treatments throughout the study period (see Table 4.6), but during the first 3 wk postpartum (when the treatments were kept at different stocking densities) the CON animals spent more time feeding after milking (see Table 4.7 on page 116).

Table 4.6 Descriptive statistics 24-h time-budget
Median time [h/d; median (IQR)] performing different behaviours in the home-pen, measured with 5-min instantaneous scan sampling. Primiparous cows were kept in either a low-stocked pen with familiar conspecifics of the same age (LS) or in fully stocked pens mixed with multiparous animals (CON) after calving. After 3 wk both treatments were housed in fully stocked pens mixed with older animals.

Behaviour	Trt	Day 1-3[1]	Day 7	Day 21[2]	Day 22-24[3]
Feeding	LS	3.47 (3.17-3.86)	4.17 (3.52-4.44)	3.88 (3.52-4.29)	5.19 (4.66-5.35)
	CON	3.39 (2.97-3.72)	4.21 (4.02-4.54)	4.75 (4.54-5.25)	4.97 (4.62-5.25)
Standing or walking					
Feed alley	LS	3.19 (2.28-3.59)	2.46 (1.83-3.81)	2.25 (1.98-2.54)	2.54 (1.88-3.15)
	CON	1.72 (1.39-2.01)	2.13 (1.65-2.44)	1.83 (1.50-2.21)	1.96 (1.65-2.24)
Back alley	LS	2.95 (2.60-4.10)	1.92 (1.10-2.60)	1.21 (1.08-2.44)	1.49 (1.17-2.84)
	CON	3.17 (2.28-4.42)	2.13 (1.69-3.50)	2.25 (1.46-3.38)	2.14 (1.67-3.03)
Lying	LS	9.42 (7.57-10.3)	9.29 (7.58-10.6)	11.7 (9.52-12.3)	9.90 (8.64-11.5)
	CON	9.32 (7.67-11.3)	9.67 (9.15-10.8)	10.7 (10.0-11.5)	10.8 (9.32-11.8)
Perching[4]	LS	2.33 (1.51-3.34)	3.08 (2.06-4.17)	3.00 (1.48-3.35)	1.92 (1.49-2.45)
	CON	3.47 (0.86-4.17)	2.13 (1.54-3.17)	1.25 (0.88-2.79)	1.68 (0.95-2.40)
Four feet[5]	LS	0.47 (0.24-0.90)	0.54 (0.29-1.44)	0.33 (0.27-0.63)	0.40 (0.31-0.67)
	CON	0.71 (0.49-0.85)	0.58 (0.29-0.79)	0.58 (0.42-0.92)	0.53 (0.33-0.81)

[1]Day in relation to calving.
[2]On this day video for two LS and one CON animal was missing.
[3]First 3 d in the second postpartum study pen; during this time all animals were mixed with multiparous cows.
[4]Standing with the front feet in the stall.
[5]Standing with all feet in the stall.

While both groups spent several h/d standing idle or walking in the alleyways, LS animals spent more time standing in the feed alley and CON animals spent more time doing so in the back alley (see Table 4.6 on page 115). Animals from the two treatments spent similar time lying during the first week after calving (Table 4.6). Compared to the last day in the post-calving pen, the LS animals had on average lower 24-h lying time after regrouping to the second postpartum pen; this change was not observed for the CON animals (Table 4.6). The majority of the animals spent >1 h/d perching (Table 4.6), but we seldom observed perching after milking (Table 4.7).

Table 4.7 Descriptive statistics post-milking time-budget
Median time [h/post-milking period; median (IQR)] performing different behaviours in the home-pen for 90-min after milking, measured with 5-min instantaneous scan sampling. Primiparous cows were kept in either a low-stocked pen with familiar conspecifics of the same age (LS) or in fully stocked pens mixed with multiparous animals (CON) after calving. After 3 wk both treatments were housed in fully stocked pens mixed with older animals.

Behaviour	Trt	Day 1-3[1]	Day 7	Day 21[2]	Day 22-24[3]
Feeding	LS	0.36 (0.30-0.41)	0.46 (0.32-0.64)	0.46 (0.38-0.63)	0.42 (0.30-0.50)
	CON	0.45 (0.35-0.50)	0.50 (0.41-0.64)	0.75 (0.56-0.83)	0.44 (0.33-0.71)
Standing or walking					
Feed alley	LS	0.35 (0.27-0.45)	0.33 (0.25-0.51)	0.21 (0.21-0.36)	0.23 (0.20-0.27)
	CON	0.19 (0.14-0.24)	0.27 (0.21-0.35)	0.17 (0.17-0.22)	0.20 (0.16-0.25)
Back alley	LS	0.12 (0.07-0.24)	0.04 (0.00-0.08)	0.08 (0.01-0.16)	0.13 (0.09-0.19)
	CON	0.24 (0.21-0.29)	0.04 (0.00-0.09)	0.04 (0.00-0.08)	0.06 (0.04-0.09)
Lying	LS	0.50 (0.20-0.69)	0.52 (0.31-0.67)	0.71 (0.31-0.78)	0.63 (0.55-0.71)
	CON	0.48 (0.27-0.60)	0.50 (0.28-0.71)	0.50 (0.42-0.71)	0.68 (0.35-0.82)
Perching[4]	LS	0.06 (0.05-0.07)	0.04 (0.00-0.05)	0.02 (0.00-0.08)	0.04 (0.01-0.05)
	CON	0.15 (0.06-0.23)	0.06 (0.00-0.08)	0.00 (0.00-0.04)	0.03 (0.01-0.08)
Four feet[5]	LS	0.02 (0.00-0.05)	0.00 (0.00-0.04)	0.00 (0.00-0.00)	0.02 (0.00-0.06)
	CON	0.01 (0.00-0.03)	0.02 (0.00-0.05)	0.00 (0.00-0.06)	0.03 (0.00-0.05)

[1]Day in relation to calving.
[2]On this day video from two LS and one CON animal was missing.
[3]First 3 d in the second postpartum study pen; during this time all animals were mixed with multiparous cows.
[4]Standing with the front feet in the stall.
[5]Standing with all feet in the stall.

Most cows in this study spent little time standing fully in the stalls, both when measured over 24-h (see Table 4.6 on page 115), and after milking (see Table 4.7 on page 116). However, extreme values did occur, and the time performing the behaviour was non-randomly distributed among the animals. Animals that were standing fully in the stall for >1 h/d on multiple days (LS: four animals; CON: four animals) had a median body weight of 600 kg, compared to 625 kg for the other animals.

4.4 Discussion

As expected, the level of social competition was higher when the heifers were housed with multiparous cows at a higher stocking density. Despite being exposed to more aggression, CON animals did not spend more time standing or perching post-calving than LS animals. In contrast to previous research (e.g. O'Connell et al., 2008; Boyle et al., 2012), we did not observe excessive standing times after mixing with older cows. In these previous studies, first-calving animals were either housed in 3-row pens with >50% of the stalls being head-to-head (all studies performed in the same facilities; O'Connell et al., 2008; Boyle et al., 2012, 2013) or were mixed with horned multiparous cows (Wagner et al., 2012); both factors could have resulted in higher social stress after regrouping. In the current study all stalls were head-to-wall, which may have made entering the stalls less aversive. The total time standing in the current study was similar to what has been reported for newly calved heifers in another study performed in the same facility (14.9 h/d; Neave et al., 2017). Even though the average standing time was unremarkable, the variability within groups was large and some animals in the two treatments stood ≥80% of the day. This variability may indicate that the animals had different strategies for coping with social stress, or that the need for rest differs between animals.

The higher rate of reactor events post-calving suggests that regrouping was more socially stressful for the CON animals. The lower stocking density in the LS pen likely allowed for larger distances between the animals at the feed-bunk, reducing the exposure to aggressive behaviours (DeVries et al., 2004). After regrouping at d 21 the rate of reactor events was similar for the two treatments, providing little evidence that the CON animals were more successful in avoiding aggression after being housed with older cows for the previous 3 wk.

Although the rate of actor events was low, at least one actor event was recorded for all study animals (both LS and CON cows) during the time they were mixed with multiparous cows. Wierenga (1990) reported that contradictory displacements (dominant cow displaced by subordinate animal) increased when linear feed space/cow decreased. Similarly, a larger proportion of animal pairs with contradictory displacements have been observed when the stocking density at the feed-bunk increased (Val-Laillet et al., 2009). It is possible that the higher number of animals in the pens containing multiparous cows forced the study animals to occasionally perform actor behaviours to gain access to feed. Conversely, the behaviour may not have been necessary in the low-stocked post-calving pen, where the feed was more likely to be freely accessible at all times.

We found similar numbers of animals with sole lesion severity score ≥3 in both treatments at wk 6 (LS: 20% vs. CON: 30% of the animals) and wk 12 (LS: 50% vs. CON: 63% of animals). The number of animals with white line lesion scores ≥3 tended to be fewer in the LS (20% of the animals), than in the CON treatment (50%) at wk 6; a *P*-value = 0.1 despite such a large effect size suggests that our study was underpowered for this outcome (Betensky, 2019). Although the low number of animals with sole and white line haemorrhages of severity 4 and 5 prevented meaningful statistical analysis, all but one animal with lesion score ≥4 were CON animals. We acknowledge that low sample size makes these results inconclusive. The results align with our hypothesis, and we argue that appropriately powered studies evaluating the relationship between the social environment and claw health are warranted. The observed effect size in this study provide some information that can guide sample size calculations in future research.

The relationship between social status and claw health have long been discussed (Galindo and Broom, 2000), and recently Newsome et al. (2016) proposed that social competition could increase the mechanical strain on the claw. We performed separate analyses for the rate of social interactions, actor events and reactor events d 1 to d 3 postpartum, to explore if the amount of aggression at the feed-bunk was related to CHDL, or if the risk depended on if the study animals performed or received aggressive behaviours. We found no relationship between the rate of social interactions or reactor events and CHDL. For most of the animals >80% of the social interactions were reactor events during this period, which explains the similar results. As many of the heifers were displaced by light physical contact, it is likely that reactor events were not a major source of mechanical strain on their claws.

The strong negative relationship between actor events and sole lesions at wk 12 is interesting, and aligns with Proudfoot et al. (2010) who reported that animals with approximately equal number of actor and reactor events around calving were least likely to develop severe sole lesions in peak lactation. The observed relationship in our study is likely not causal, but rather suggests that heifers that perform actor behaviours the days following calving are better able to succeed in a competitive environment. This would explain why no association was found at wk 6, as only half of the animals had been housed in a competitive environment >3 wk at that time.

We found a large variability between the animals for all behaviours, with the exception for time spent feeding. The lower variability for feeding may be explained by the high motivation of lactating dairy cattle to access feed (Schütz et al., 2006). CON animals generally spent more time feeding after milking in the post-calving pen than did LS animals. Proudfoot et al. (2009) reported that primiparous cows overstocked at the feed-bunk had longer periods of sustained activity at the feeder (i.e. meals) than did primiparous cows with one feed space/animal; this difference was due to longer intervals between individual visits to the feeder within the same meal. Hence, it is possible that the longer feeding times for the CON animals were a result of more frequent displacements at the feed-bunk after milking in the fully stocked control pens.

It is interesting to note that the LS animals spent several hours each day standing in the alleyways, despite having free access to deep-bedded sand stalls of adequate size. To reduce the mechanical load on the claws, it may be necessary to provide both comfortable lying and standing spaces within the home-pen (Fregonesi et al., 2004; Lobeck et al., 2011). Cows in the two treatments spent similar time standing idle, but LS animals spent a larger part of the day standing in the feed alley and CON animals spent more time in the back alley between the stalls. One explanation for this observation is that CON animals may have chosen to remain in the more crowded back alley to have easier access to a preferred free-stall when this became available. Better ability to compete for stalls could explain why the CON animals maintained their 24-h lying time after regrouping at d 21, while the average lying time of the LS animals decreased.

Low-ranked animals may hide from dominant cows by standing fully or partially in the stalls (e.g. Wierenga and Hopster, 1990), but our observations provide little support for this idea. Little time was spent perching and standing in the stall after milking, which presumably is a period with more social competition. When they were perching, the animals often stood with their front feet on the back curb, leaving most of their body located in the back alley, and animals were also seen standing with their front feet on elevated surfaces in the alleyways. More efforts should be made to understand why this behaviour is so common.

Except for a few animals in both treatments, little time was spent standing fully in the stalls. The lower body weights of these animals suggest that only the smaller animals could comfortably stand fully in these stalls, taking advantage of the soft standing surface (Fregonesi et al., 2009a).

4.5 Conclusions

Under the conditions of this study regrouping did not lead to prolonged standing time or increased time perching in newly calved primiparous cows. It was uncommon for the animals to perform actor behaviours at the feed-bunk after regrouping, while aggression directed towards the study animals occurred less frequently in the low-stress social environment. Animals that more frequently performed actor behaviours at the feed-bunk the days following calving were less likely to have sole lesions at wk 12.

Chapter 5: General discussion and conclusions

5.1 Thesis findings

The main objective of my thesis was to contribute to the understanding of how standing behaviour relates to the development of CHDL, with a particular focus on the transition period. My research also investigated the effect of the social environment on standing behaviour and claw health.

My review of the literature provided insight into the many factors that can affect standing behaviour, and emphasised that cows with low social status often change their behaviour to a larger degree during periods of increased social competition. First-calving animals are often socially subordinate; yet few studies have recorded their behavioural responses to social stress, nor how their standing behaviour affects claw health. Despite the recent interest in the transition period as a time of increased risk for lameness and CHDL, only three studies have evaluated the relationship between standing behaviour during the transition period and CHDL. These studies were all performed at the same research facility, limiting the external validity of the findings. These gaps have been addressed in my empirical chapters, and other important gaps in the literature will be discussed in section 5.2.4.

One of the challenges I faced in my empirical research was how to interpret longitudinal locomotion data. There are currently no recommendations for how often locomotion should be assessed to identify new cases of lameness in longitudinal studies, and how to account for observer inconsistency. Therefore, I assessed how the case definition and the assessment frequency affected measures of lameness incidence in Chapter 2. I found that using a single observation of compromised locomotion to identify lameness was poorly related to more strict lameness definitions, and to the presence of claw horn lesions and infectious claw lesions at trimming. I also found that when animals were assessed every 3 wk, many lameness events remained undetected. These results will help guide future longitudinal lameness research, and may have practical implementations for daily farm management.

In Chapter 3, I evaluated how daily standing time and long standing bout duration during the transition period were related to sole lesions later in lactation. I found that prolonged standing, and long standing bouts during the first 2 wk of lactation were associated with a higher odds of developing sole lesions. Long standing time during the transition period has been related to sole lesions in previous experimental studies, and the work presented in this thesis strengthen the evidence that the association is generalizable. Additionally, the observed relationship between standing bout duration postpartum and sole lesions in peak lactation is a novel finding, which suggests that management practices that result in long standing bouts can negatively affect claw health.

In Chapter 4, I evaluated if newly calved heifers regrouped to a low-stress social environment would spend less time standing and perching than animals mixed with multiparous cows. No difference was found between treatments for these behaviours, and in contrast to previous research I did not observe prolonged standing in either treatment. This unexpected finding suggests that behavioural responses to regrouping are context dependent. Further research is needed to determine what factors can mitigate social stress after regrouping.

In the remainder of this chapter, I describe some of the limitations of my work and provide suggestions for future research.

5.2 Limitations and future research directions

5.2.1 Lameness assessment

A large number of different locomotion scoring scales are used in lameness research, reflecting how challenging it is to correctly identify if an animal is lame (see Schlageter-Tello et al., 2014b for a comprehensive overview of scoring systems reported in the peer-reviewed literature). Evaluation of the different scoring systems has often focused on how reliably an animal can be identified as lame by multiple observers, or if the same observer repeatedly can score the animal as lame. Given that most evaluations report imperfect reliability (e.g. Bicalho et al., 2007; Thomsen et al., 2008; Schlageter-Tello et al., 2015), it is not clear how much emphasis should be placed on an individual gait score, making interpretation of longitudinal locomotion data challenging. However, multiple measurements do provide more information, which may be used to more reliably identify animals that need treatment.

The study presented in Chapter 2 is the first to evaluate how the case definition and assessment frequency affect measures of lameness incidence in longitudinal locomotion data. In preparation for this study, two observers were jointly trained in locomotion scoring. While almost perfect reliability was achieved when assessing locomotion from video, the inter-observer reliability was moderate for live scoring. This notable divergence was likely a result of multiple factors that differed between video and live training. Video recordings provided the same visual input to both observers, something that was not possible to achieve when performing live scoring. Even when the observers simultaneous scored the same cow, they did not watch the animal from exactly the same angle, and therefore based their decision on different information. In addition, during video training a similar number of animals per gait score were presented. This is a reasonable approach, given that the goal of the training was to improve and align the observers' ability to differentiate between different locomotion scores. However, during live scoring the majority of the animals (>95%) either had imperfect gait or were mildly lame, the two categories most difficult to distinguish (Schlageter-Tello et al., 2014a). Substantial differences in prevalence between categories in known to decrease kappa estimates (the prevalence paradox; Sim and Wright, 2005), so the low number of severely lame animals (<5%) may have contributed to the lower kappa-value for live scoring.

A practical limitation of the work described in Chapter 2 is that we assessed the locomotion of the animals on a weekly basis. Although this sampling regime was quite frequent compared to other longitudinal lameness studies (weekly to monthly intervals; e.g. Bicalho et al., 2007; Frankena et al., 2009; Randall et al., 2017), it differs greatly from the daily (albeit unsystematic) assessments reported for lactating animals by many of the farmers in Chapter 3. The animals enrolled in the study were also followed for a relatively short period (approximately 2 mo), which prevented us from evaluating the effect of assessment intervals longer than 3 wk. It would be interesting to evaluate the usefulness of different case definitions over a wider range of assessment intervals.

Given the labour needed to identify and treat lame animals, I encourage the development of automated lameness detection methods. Although a laudable goal, the technical development and practical implementation on commercial farms still pose many challenges (reviewed by Van Nuffel et al., 2015). Optimally, automated locomotion scoring systems should enable early detection and treatment of lameness (Thomas et al., 2015), without unduly increasing the work load for the farm staff.

5.2.2 Standing behaviour

The study presented in Chapter 3 provided the first evidence that prolonged standing during the transition period is associated with sole lesions later in lactation on commercial farms. The observational nature of the study makes it difficult to make inferences beyond a temporal association between the exposure and the outcome (Dahoo et al., 2009). However, the study design and the associated statistical analyses were carefully developed to control for other factors known to affect the risk of developing sole lesions. That said, as the animals were not randomly assigned to the risk exposure, there is a possibility that unidentified confounders may have influenced the observed relationship (Dahoo et al., 2009). For example, as the animals were not followed from young age, it is likely that at least some animals with previous CHDL were included in the data set. This opens up the possibility that older pathological changes in the internal structures of the claw could cause pain by increasing the pressure on the corium during the time period when the laxity of the suspensory apparatus was increased. The pain could then have made the animals more reluctant to lie down (Cook et al., 2004), increasing the time the animals spent standing (i.e. reverse causation). However, this pain would presumably have made the animals more reluctant to stand up, resulting in long standing bouts and decreased standing time for animals with previous CHDL. Compared to sound cows, most previous research has reported that lame animals spent more time lying down (Singh et al., 1993; Calderon and Cook, 2011; Solano et al., 2016), including moderately lame animals (Weigele et al., 2018). As such, if the relationship between standing behaviour around calving and sole lesion in peak lactation was confounded by pre-existing pathology, it seem plausible that I would have observed a positive association between sole lesions and average longest standing bout, and a negative relationship between sole lesions and average standing time.

Although randomised clinical trials provide evidence of the highest scientific rigor, it is also important to consider the external validity of the findings (discussed by Steckler and McLeroy, 2008). The results presented in Chapter 3 support the findings from earlier studies conducted at the UBC Dairy Education and Research Centre, and provide evidence that the association between prolonged standing and sole lesions is generalizable.

A number of limitations that were present in this study are worthy of discussion. Most notably the time spent perching could not be recorded due to practical constraints. In the study of Proudfoot et al. (2010) prolonged standing was confounded with time spent perching, and Dippel et al. (2011) reported that perching in early lactation was associated with the development of sole lesions later in lactation. These results suggest that there is a need to disentangle time spent perching from other standing measures when evaluating the effect of prolonged standing. To date there is no equipment available that can automatically determine if the cow is standing partly in the stall, as opposed fully outside or inside the stall. As such, 24-h video assessment would have been necessary to record time spent perching for the enrolled animals; however, the large number of cows on different farms made this unfeasible in the study presented in Chapter 3.

Another study limitation is the method used to record claw lesions. Although it would have been better if claw lesions had been scored by the same trained observer, and recorded in accordance with a lesion severity scale previously used in research, this was not possible due to time constraints. The benefits of using two certified trimmers from the same company were that they used the same scoring method, that both had extensive experience in lesion assessment, and that lesions were recorded by observers blind to our locomotion data. Disadvantages were that practical and financial considerations restricted the possibilities to obtain formal reliability measures, and that mild haemorrhages were not recorded. As a result, the number of animals with recorded CHDL was lower than predicted, which restricted the types of analyses that could be performed.

An obvious follow-up question from Chapter 3 is why certain cows spent a long time standing during the transition period, and I encourage future research to address this question. Given the reviewed research presented in Chapter 1, there are likely multiple reasons for prolonged standing during this period, and the different reasons may require different types of intervention to reduce the risk of sole lesions.

5.2.3 Social environment

The biggest contribution of the work evaluating the effects of the social environment on standing behaviour (Chapter 4) is the questions that were identified, rather than the answers it provided. It is unclear why prolonged standing was not observed after mixing newly calved heifers with multiparous cows at 100% stocking density. Other studies using similar stocking have reported excessive standing times after heifers were mixed with older cows (O'Connell et al., 2008; Wagner et al., 2012; Boyle et al., 2013). As was suggested in the empirical chapter, it would be worthwhile for future research to evaluate if the pen configuration influences the animals' behavioural response to regrouping, and explore what environmental factors can mitigate prolonged standing. The increased use of activity monitors on commercial farms may make this research more feasible.

An important limitation of the study is that given the available sample size, an unreasonably large effect of the social environment on the risk of developing CHDL (>40 percentage points difference between the treatments) would have been necessary to obtain a *P*-value below the significance level most commonly used within the field ($P < 0.05$). A larger sample size would certainly have been beneficial, but the high level of environmental control necessary to perform this study would have made the project impractical on larger commercial operations. Even at the UBC Dairy Education and Research Centre the study animals could only be kept in the low-stocked treatment during the summer, illustrating the practical restrictions that empirical research often faces. The current study provided a unique opportunity to evaluate if a low-stress social environment during the transition period would reduce the risk of CHDL. Although the results should be considered preliminary, they provide information that can help guide future research. More efforts should be made to investigate if the housing strategies that are currently considered good industry practice can negatively affect claw health.

The negative relationship between the rate of actor events and sole lesions at wk 12 postpartum suggests that how primiparous cows respond to their social environment may influence claw health, but the causal relationship is unclear. Newsome et al. (2016) suggested that social competition could result in inappropriate forces damaging the corium, but no association was found between the rate of social interactions (actor and reactor events combined) at the feed-bunk and CHDL in the current study. To better understand the mechanical forces on the hoof caused by different types of aggressive interactions, future research could use pressure mats to evaluate the association with aggressive interactions recorded from video. This method could also be used to evaluate the relationship between the stocking density (high stocking density is known to increase the number of aggressive interaction; Olofsson, 1999; Fregonesi and Leaver, 2002; DeVries et al., 2004) and the accumulated mechanical strain on the hoof.

5.2.4 Other literature gaps for future research consideration

Other gaps in the literature warrant mentioning, despite not being directly related to the association between standing behaviour and CHDL. I have identified three important areas for future research: 1) periparturient physiology, 2) etiopathogenesis of CHDL, and 3) functional adaptation of the protective structure in the claw.

Much still remains to be understood regarding CHDL development. For example, it is still unknown if the increased laxity in the suspensory apparatus during the transition period is caused by an increase in circulating hormones around calving (Tarlton et al., 2002), or if it is a result of other factors present during this time period (Newsome et al., 2017b). Similarly, how long the biomechanical properties of the suspensory apparatus are altered after calving is unclear, though the available evidence indicates that the laxity is still increased 12 wk postpartum (Tarlton et al., 2002).

Although multiple studies have found an association between CHDL and the amount of new bone formation (osteophytes) on the pedal bone (Tsuka et al., 2012; Newsome et al., 2016) the causal relationship is unclear. By using portable x-ray equipment it may be possible to determine the temporal association, and to investigate if the risk for recurrent CHDL is related to the size of the osteophytes.

Another promising line of research is investigating if management during early life affects the functionality of the protective structures in the claw. Although the study was poorly replicated, Gard et al. (2015) reported an interesting positive association between the amount of exercise in the post-weaning period and the size of the digital cushion at six months of age in dairy bull calves. I encourage more exploration of this topic, including potential long-term effects of exercise.

5.3 General conclusions

The findings of my thesis show that how longitudinal lameness studies are designed, and the data are interpreted, can affect their conclusions. The results presented in Chapter 2 suggest that with sufficient training, observers can become skilled in differentiating between different gaits scores, and remain proficient for at least six months. However, the results also indicate that it is difficult to differentiate between GS2 and GS3, and that observer inconsistency should be accounted for when interpreting longitudinal data. The work described in Chapter 3 contributes to the knowledge on how standing behaviour during the transition period relates to sole lesions during peak lactation on commercial farms. The findings in Chapter 4 illustrate that the social environment can affect the behaviour of newly calved heifers, and suggest that how the animals respond to social stress may alter their risk of developing claw health. By employing longitudinal study design I have been able to address three key gaps in the literature, and collectively the work presented in this thesis illustrates the value of this type of studies when evaluating risk factors for claw horn lesions.

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