



## Standing behavior and sole horn lesions: A prospective observational longitudinal study

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### ABSTRACT

Studies performed on individual research farms have reported that dairy cattle developing sole hemorrhages or sole ulcers in peak to mid lactation spent more time standing during the weeks around calving. The aim of this prospective observational longitudinal study was to evaluate whether this relationship is evident in commercial dairy herds. A convenience sample of 8 herds were visited every other week, and animals without previous severe horn lesions and deemed sound at 4 to 8 wk before calving were enrolled. Standing behavior was measured with data loggers attached to a rear leg, and standing time and duration of the longest standing bout were determined for each cow. Standing behavior was summarized into 3 periods: before (d −14 to −2), around (d −1 to 1), and after (d 2 to 14) calving. Average daily standing time and average daily longest standing bout were determined for each cow and period. Average daily standing time was normally distributed, with a mean  $\pm$  standard deviation of  $12.1 \pm 1.6$ ,  $14.4 \pm 2.2$ , and  $13.8 \pm 1.7$  h/d for the 3 periods, respectively. Average daily longest standing bout was right skewed with a median of 3.6 h/d [interquartile range (IQR): 3.0 to 4.3; range: 1.7 to 12.1], 3.9 h/d (IQR: 3.1 to 4.8; range: 1.3 to 11.5), and 3.7 (IQR: 3.2 to 4.4; range: 1.5 to 11.7) h/d before, around, and after calving, respectively. Hoof trimming was performed 8 to 12 wk postpartum; hoof lesion data were summarized per cow, and the most serious injury of each type of lesion was noted. Sole hemorrhages or sole ulcers were found in 25 of 256 cows. Mixed-effect logistic regression models with herd as random effect were used to analyze the risk of developing sole hemorrhages and sole

ulcers, using animals without hoof lesions as reference category. Separate models were fitted for the 2 standing behaviors, and for the periods before, around, and after calving. Change in standing behavior from before to after calving was also analyzed. Body condition score at calving, body condition score loss in early lactation, milk yield, parity, and days in milk at trimming were included as covariates. In this study, no evidence for an association was found between sole hemorrhages and sole ulcers and standing behavior before or around calving. Longer standing time and longer standing bouts after calving were associated with increased odds of developing sole hemorrhages and sole ulcers, as was an increase in standing bout duration from before to after calving. Animals with sole horn or white line lesions had higher unconditional sample odds of becoming lame (odds ratio = 2.5) and severely lame (odds ratio = 11.7) after calving, compared with animals with no registered lesions at trimming. Multiparous animals had higher lameness incidence, both before and after calving. Avoiding practices that exacerbate increases in standing time and standing bout duration in early lactation may reduce the incidence of sole hemorrhages and sole ulcers.

**Key words:** hoof pathology, longitudinal study, standing bout

### INTRODUCTION

Previous research has found that cows that spend more time standing are more likely to develop sole hemorrhages and sole ulcers (Singh et al., 1993; Galindo and Broom, 2000) and become lame (Galindo and Broom, 2000). The transition period [i.e., 3 wk before to 3 wk after calving (Grummer, 1995)] may be particularly important for hoof health. During this time the laxity in the hoof's supportive structure is increased, which is believed to increase the risk of mechanical damage within the hoof capsule (Tarlton et al., 2002; Knott et al., 2007). It has also been suggested that mobilization of body fat during early lactation can result in thinning of the digital cushion (Bicalho et al., 2009). Thinning of the cushion reduces its force dissipating capacity,

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further increasing the risk of horn lesions (Räber et al., 2004). In a prospective cohort study, Newsome et al. (2017a) reported that animals with little soft tissue between the sole horn and the pedal bone had increased risk of future horn lesions. A companion paper (Newsome et al., 2017b) reported that the sole soft tissue was thinnest the week after calving, further supporting that the risk of hoof damage increases during the transition period.

A positive relationship between standing time during the transition period and sole hemorrhages or sole ulcers has been reported in multiple studies (Chapinal et al., 2009; Proudfoot et al., 2010). However, these studies were performed under controlled conditions on one university research farm, limiting the extent to which results can be generalized. Additionally, previous literature has focused on the effects of standing time; the relationship between standing bout duration and sole hemorrhages or sole ulcers was only assessed for average bout duration (Proudfoot et al., 2010). When dairy cows are forced to stand, behaviors 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 behaviors suggest that long standing bouts are uncomfortable for the cow; it thus may be more biologically relevant to evaluate the effect of the longest daily standing bout rather than the average bout duration.

Here we report the results of the first prospective longitudinal study on commercial dairy herds evaluating what aspects of standing behavior during the transition period are related to the development of sole hemorrhages and sole ulcers later in lactation. We hypothesized that dairy cows with increased standing time and long bouts of standing would be at higher risk of developing sole hemorrhages or sole ulcers during early lactation. Based on Proudfoot et al. (2010), who reported that the relationship between standing time and sole hemorrhages and sole ulcers differed pre- and postpartum, we split standing behavior into 3 periods: before ( $d -14$  to  $-2$ ), around ( $d -1$  to  $1$ ), and after ( $d 2$  to  $14$ ) calving. Secondary study aims were to describe standing behaviors during the transition period, and to assess postpartum gait in relation to postpartum hoof trimming findings.

## MATERIALS AND METHODS

### Herd Selection

This study was approved by the Animal Care Committee at the University of British Columbia (UBC; protocol A15-0084). A convenience sample of 8 commercial dairy herds in the lower Fraser Valley region

(BC, Canada) were visited every other week between May 2017 and January 2018. The herds were recruited by partnering with a local hoof trimming company (AR-PE Hoof Trimming Ltd., Abbotsford, Canada). Herds were eligible for enrollment if the herd size was  $\geq 160$  lactating animals, dry cows and pre-calving heifers were kept separately from lactating animals, and animals were predominantly housed in freestall pens (the use of deep-bedded open packs for a short period in the end of the dry period was acceptable). Additional herd inclusion criteria included that farmers used herd management software with electronically extractable data, recorded calving date and milk yield for individual animals, and performed regular trimming at least once per lactation. Herds were contacted by phone based on proximity to the UBC Dairy Education and Research Centre (Agassiz, BC, Canada). Of 13 contacted herds, 9 agreed to participate in the study; 1 herd was excluded shortly after enrollment because the configuration of the dry pens impeded data collection.

During the initial herd visit a structured interview was conducted with the owner or manager to collect information about herd characteristics (Table 1) and hoof health management (Table 2). On the same occasion, an environmental assessment was performed in the pre- and postpartum pens, walkways, waiting area for milking, and parlor (Table 3).

### Sample Size

A power analysis was undertaken, based on reported sole hemorrhage and sole ulcer 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 sole horn lesions previously (Leach et al., 1997). A 20% difference in the number of animals developing sole hemorrhages and sole ulcers was considered biologically relevant (prevalence 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 herds, this estimate was considered conservative, so we set our target enrollment at 300 cows.

### Study Design

Based on previous research, a causal diagram was used to identify cow-level variables affecting the risk of sole hemorrhages and sole ulcers (Figure 1). Only animals with complete trimming records were eligible for enrollment; in 1 herd this excluded parity 5+ animals. Multiparous cows with less than one trimming per lactation were not included in the study. Animals

with no previous record of severe horn lesion (i.e., cows that had never been diagnosed with sole ulcer, thin soles, toe ulcer, periople ulcer, or white line disease), and deemed sound [locomotion scoring (**LS**) <3] at first assessment 4 to 8 wk before expected calving, were enrolled on an ongoing basis. The animals were assessed every other week until approximately 8 to 12 wk postpartum, when they were hoof trimmed as part of the study. The trim period was chosen to allow for sole hemorrhages and sole ulcers developing during the transition period to become visible on the sole surface (Kempson and Logue, 1993). Expected and actual calving date, and parity were retrieved from the herd management software.

### Standing Behavior

To measure standing behavior, enrolled animals were fitted with electronic data loggers (HOBOPendant G Acceleration Data Logger; Onset Computer Corp.) on one of their rear legs, approximately 7 to 10 cm above the fetlock. Standing behavior was recorded from approximately 4 wk before expected calving until approximately 2 wk postpartum, and loggers were

changed every other wk. Loggers were attached so the y-axis pointed upwards, and were set to record *g*-force acceleration for the y-axis once per min.

Logger data were downloaded using HOBOWare (Onset Computer Corp.) and raw *g*-force values were exported as CSV files, which were imported into R version 3.4.4 (RStudio Team, 2016; R Core Team, 2018) 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 (Ledgerwood et al., 2010) cut-off of 2.55 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 represented the orientation of the rear leg at the moment of recording, which means that, for example, 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 zeroes to 0, and vice versa (2-min filter; Ledgerwood et al., 2010). The filtered data were summarized to determine standing time (min) and the duration of the longest standing bout (min) for each

**Table 1.** Herd characteristics and management on 8 dairy herds in the lower Fraser Valley region of British Columbia, Canada

Herd	A	B	C	D	E	F	G	H
Herd size <sup>1</sup>	185	510	540	310	330	290	250	610
305-d milk yield (kg)	12,700	12,800	12,900	10,500	9,100	12,200	12,800	13,000
Breed	Holstein	Holstein	Holstein	Holstein	Mixed <sup>2</sup>	Holstein	Holstein	Holstein
Geographical sites	1	3 <sup>3</sup>	1	2 <sup>4</sup>	1	1	1	2 <sup>4</sup>
Milkings per day	2	3	2	2	2	2	3	2–3 <sup>5</sup>
Feedings per day								
Dry animals	1	1	1	1	0.5 <sup>6</sup>	1	1	1
Lactating animals	1	1	1	2	1	2	1	1
Cows per feed space <sup>7</sup>								
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 per lying space <sup>8</sup>								
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, dry-off to early lactation	5	5	5	5	4–5 <sup>9</sup>	6	5	6
Introduction to main herd <sup>10</sup> (wk)	–3	–3	–2	–8	–6	0	–8	–5
Separate lactating pen for parity 1	No	Yes	Yes	Yes	Yes	No	Yes	Yes <sup>11</sup>

<sup>1</sup>Sum of dry and lactating animals; pregnant heifers are not included.

<sup>2</sup>Animals cross-bred with Holstein, Ayrshire, and Jersey.

<sup>3</sup>Dry animals kept on separate farm in the same district; heifers kept on dry-lot in another district.

<sup>4</sup>Heifers kept on dry-lot in another district.

<sup>5</sup>Herd changed to 3 milkings per day midway through the study period.

<sup>6</sup>Dry cows fed every other day.

<sup>7</sup>One feed space is defined as either one head-lock, or 60 and 76 cm of linear feed space for lactating and dry cows, respectively. Values represent average number of cows per feed space during the study period.

<sup>8</sup>One lying space is defined as either one freestall or 11 m<sup>2</sup> in open-pack lying areas. Values represent average number of cows per lying space during the study period.

<sup>9</sup>Primiparous cows = 4 pen-changes; multiparous cows = 5 pen-changes.

<sup>10</sup>Time point when first-calving animals were mixed with dry or lactating multiparous cows, measured by weeks in relation to first calving.

<sup>11</sup>Until confirmed pregnant, then mixed with multiparous cows.

**Table 2.** Lameness prevalence and hoof health management on 8 dairy herds in the lower Fraser Valley region of British Columbia, Canada

Herd	A	B	C	D	E	F	G	H
Prevalence % (severe) <sup>1</sup>	42 (7)	27 (3)	55 (25)	32 (3)	30 (7)	32 (11)	21 (3)	25 (3)
Footbaths/wk	2	1	2	4	1	0	3	2
Solution <sup>2</sup>	F	F	F/Zn <sup>3</sup>	Cu/Zn <sup>4</sup>	F	—	F	F/Zn <sup>5</sup>
Routine trimmings per lactation	1–2	2	2	3	Ad hoc <sup>6</sup>	1	2	1
Locomotion assessment frequency	Weekly <sup>7</sup>	Daily <sup>8</sup>	Daily <sup>8</sup>	Daily <sup>8</sup>	Daily <sup>8</sup>	Daily <sup>9</sup>	Daily <sup>8</sup>	Daily <sup>8</sup>
Lactating cows	Weekly <sup>7</sup>	—	—	2 times/wk	—	Daily <sup>9</sup>	—	—
Dry cows	TL	TL	—	TL	TL	TL	O	O
Management acute lameness <sup>10</sup>	AT, O	AT, O, C, P	AT	AT, O, C, P	O	O	O	O, C
Mild	P	L, TR	L, TR	L, TR	L, TR	L, X	OR	L, TR
Severe								
Follow-up <sup>11</sup>								

<sup>1</sup>Lactating herd prevalence of lameness (severe lameness) at the initial herd visit.

<sup>2</sup>F = formaldehyde; Zn = zinc sulfate; Cu = copper sulfate.

<sup>3</sup>Formaldehyde used during summer months, and acidified zinc sulfate during winter months.

<sup>4</sup>Copper and zinc sulfate used on alternating occasions.

<sup>5</sup>Formaldehyde and zinc sulfate used on alternating occasions.

<sup>6</sup>Multiparous cows that were not trimmed at least annually were not included in the study.

<sup>7</sup>Assessment performed during scheduled occasions set aside for locomotion scoring.

<sup>8</sup>Assessment performed during milking.

<sup>9</sup>Assessment performed during pen walks for identification of unhealthy animals and animals in heat.

<sup>10</sup>AT = acute trim (veterinarian or trimmer); C = moved close to parlor; O = own acute trim; P = moved to open pack; TL = moved to open pack; TR = moved to open pack; TR = recheck by trimmer next ordinary visit.

<sup>11</sup>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.** Environmental assessment on 8 dairy herds in the lower Fraser Valley region of British Columbia, Canada

Herd	A	B	C	D	E	F	G	H
Pen layout	Freestall	Freestall	Freestall	Freestall	Freestall	Freestall	Freestall	Freestall
Far-off pens	Open pack	Open pack	Freestall	Open pack	Freestall	Freestall	Freestall	Freestall <sup>1</sup>
Close-up pens	Freestall	Freestall	Freestall	Freestall <sup>2</sup>	Freestall	Freestall	Freestall	Freestall
Lactating pens	Scraper	Scraper	Flush	Tractor <sup>3</sup>	Robot	Scraper	Scraper	Scraper
Manure handling	0 (0–0)	1 (0–2)	1 (0–1)	1 (0–2)	0 (0–1)	0 (0–1)	1 (0–1)	0 (0–0)
Alley cleanliness <sup>4</sup>								
Flooring								
Far-off pens	Concrete	Concrete	Concrete	Concrete	Concrete slats	Rubber	Concrete	Concrete
Close-up pens	Concrete	Concrete	Concrete	Concrete	Concrete slats	Rubber	Concrete	Concrete
Lactating pens	Concrete	Concrete	Concrete <sup>5</sup>	Concrete	Concrete slats	Concrete	Concrete	Concrete
Walkways	Concrete	Concrete	Concrete	Concrete	Concrete slats	Concrete	Concrete	Concrete
Waiting area	Concrete	Concrete	Concrete	Concrete	Concrete	Concrete	Rubber	Concrete
Parlor	Concrete	Concrete	Concrete	Concrete	Rubber	Concrete	Rubber	Rubber
Parlor type	Herring-bone	Rotary	Parallel	Herring-bone	Parallel	Parallel	Parallel	Parallel

<sup>1</sup>Animals moved to deep-bedded open pack 7 d before expected calving.<sup>2</sup>Fresh cows housed on open pack for the first 3 to 5 d postpartum.<sup>3</sup>Scrapers present in the first-parity lactation pen.<sup>4</sup>Alley cleanliness was measured in the feed alley; 0 = manure depth up to 0.5 cm; 1 = 0.5 to 1 cm; 2 = 1 to 3 cm; 3 = more than 3 cm. On average 39 pen assessments were performed per herd; values represent median (interquartile range).<sup>5</sup>Rubber flooring was present by the feed bunk.

cow and day. Days when data loggers were attached, changed, or removed were excluded from the data set.

## Locomotion

Before data collection started, 2 observers with previous experience in LS were trained to align their ratings using an ordinal scale of 1 to 5 (LS1 to LS2 = freely able to move, LS3 = compromised locomotion, LS4 to LS5 = severely restricted ability to move; Flower and Weary, 2006). Training in identification of abnormal gait characteristics (head bob, back arch, shortened track-up, asymmetrical weight bearing, and limp), and overall LS was performed using video recordings of cows walking in a straight line on dry concrete, followed by training with live observation at the UBC Dairy Education and Research Centre.

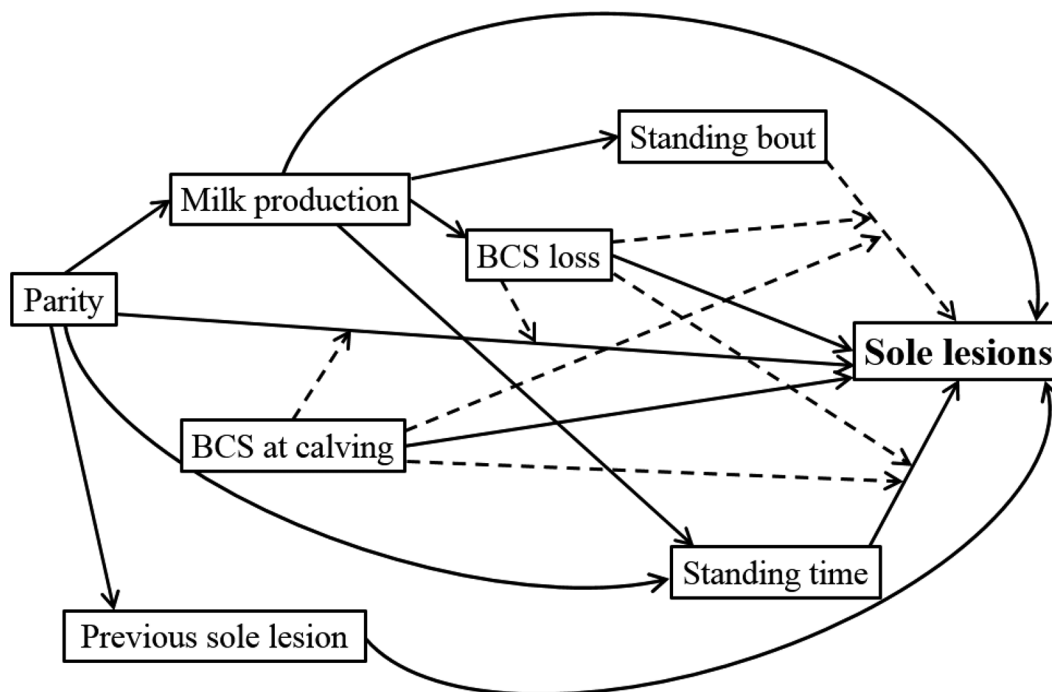
Details about the training methodology and evaluation of interobserver reliability have been described elsewhere (Eriksson et al., 2020). In brief, interobserver agreement was calculated using quadratic weighted kappa (Cohen, 1968). For video recordings, the kappa value was 0.84, and for live scoring at the beginning and end of the data collection, kappa values were 0.57 and 0.55, respectively. The bias index was used to evaluate systematic bias between the observers, resulting in values close to 0, indicating no systematic bias between the observers (Byrt et al., 1993).

The locomotion of all lactating animals was assessed as the cows exited the parlor during the initial visit on each herd, to estimate herd lameness prevalence at the beginning of the study period. During the study period, enrolled cows were locomotion scored during pen walks where unrestricted animals walked on concrete while being observed directly and obliquely from behind. Locomotion assessment of the enrolled cows was performed by 1 of the 2 observers at each visit (every 2 wk) from enrollment to the first visit after trimming. Both observers scored cows on all herds. Information about animals with  $\geq$ LS3 was given to the herds after every visit (except on those herds that specifically requested only information about  $\geq$ LS4), and treatment was performed at the discretion of the herd manager.

## Hoof Lesions

Postpartum trimming was performed by 1 of 2 certified (PTC<sup>+</sup>, Oenkerk) trimmers, belonging to the same trimming company through which herds were recruited. The trimmers routinely scored hoof lesions in accordance to the hoof lesion scale used in this study (Alberta dairy hoof health project: lesion severity scoring guide; Mason, 2014); only lesions remaining after trimming were recorded. All types of lesions were recorded per





**Figure 1.** Causal diagram of proposed cow-level factors affecting the risk of sole hemorrhages and sole ulcers. Line type indicates the level of current evidence: (1) support from peer-reviewed literature (solid lines), (2) proposed by the present study (dashed lines). Arrows between boxes illustrate direct effects, and arrows touching arrows indicate that the effect of a variable depends on the value of another variable (interaction). For example, the effect of standing bout duration on sole horn lesion risk is suggested to depend on BCS at calving.

hoof, using Hoof Supervisor System software (KS Dairy Consulting Inc.). Findings registered at trimming were obtained from paper printouts, containing individual ear-tag number, type of lesion, severity of lesion, and trimming date. A cow was considered affected with a lesion if it was found on at least one hoof, 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.

## BCS

Body condition score was measured in 0.5-point increments using a numeric rating scale from 1 to 5 (1 = very thin, 3 = good condition, 5 = obese; Ferguson et al., 1994) by 1 of 4 observers (of which 2 also scored locomotion), jointly trained before the start of data collection. Interobserver reliability was evaluated from 54 cows, scored by all observers on the same day in one of the participating herds. 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. Intraclass correlation (ICC) was used to evaluate the reliability, as it allows for evaluation of more than 2 observers of ordinal

data. As the observers were nonrandomly selected and it was important that the scores were the same between observers, the ICC was specified as 2-way with 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 4 observers ( $0.81$ ; 95% CI:  $0.73$ – $0.87$ ) indicated good to excellent agreement for BCS assessment.

The BCS measurements were taken on head-locked animals (to allow for palpation of the tail head) on regular farm visits 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.

## Milk Production

On the first biweekly visit occurring after 50 d postpartum, DIM and 24-h uncorrected milk production in kilograms (registered in the parlor) for that day were recorded for each cow using the herds' management software. For cows that lacked milk recordings at this visit (e.g., bucket milked due to medical treatment), DIM and 24-h milk production were obtained on the first visit with available milk recordings between 50 and

100 DIM. One herd used monthly milk recordings to measure milk production; for cows in this herd we used data from the first milk recording occurring during the same period.

### Data Handling and Statistical Analyses

When assessing the animals during farm visits, data were recorded on paper forms and later transcribed into an online electronic repository. The transcription error rate was assessed for 5% of the full data set, with 0.008% of the assessed measurements incorrectly entered. When mistakes were discovered during this process, the entries were corrected. For further data handling and analyses data were 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). The data sets and accompanying R scripts are available at <https://dataverse.scholarsportal.info/dataset.xhtml?persistentId=doi:10.5683/SP2/XP3JBE> (Weary et al., 2021).

### Data Handling

Animals were categorized as becoming lame if they scored LS3 on  $\geq 2$  consecutive visits or  $\geq$ LS4 at least once (Eriksson et al., 2020), with  $\geq$ LS4 being considered severely lame. Cows that became lame before calving were not included when analyzing cow-level risk factors for sole hemorrhages and sole ulcers, to further reduce the risk of including animals with previous horn lesions in this data set. These cows were also excluded when assessing postpartum gait in relation to trim findings, but they were included when assessing pre- and postpartum lameness incidence. The number of cows therefore differ between the performed analyses.

When analyzing risk factors for sole hemorrhages and sole ulcers, we used animals without any hoof lesion at trimming as the reference category; animals with other types of hoof lesions (i.e., infectious hoof lesions, white line lesions, and thin soles) were excluded from these analyses.

Standing behaviors were transformed from minutes/day to hours/day and summarized per cow by the following periods: before (d  $-14$  to  $-2$ ), around (d  $-1$  to  $1$ ), and after (d  $2$  to  $14$ ) calving. Average daily standing time, average daily longest standing bout (average length of the longest standing bout per day during the period), and the individual longest standing bout of the different periods were summarized by cow. The periods were tested separately to assess if the effect of standing differed between periods. Animals with less than 5 d of recorded standing behavior 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 analyzed. Change in standing behavior from before to after calving was calculated by subtracting the prepartum value from the postpartum, resulting in a positive value if average daily standing time or average daily longest standing bout increased after calving.

The BCS at calving was estimated using the average score from the last 2 visits before calving. The animals were dichotomized as BCS  $< 3.25$  versus BCS  $\geq 3.25$  at calving. 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, resulting in negative values if the animal lost condition and positive values if the animal gained condition during the period. Based on the results of Lim et al. (2015), who reported that animals losing  $\geq 0.75$  BCS in early lactation had increased lameness risk, cows losing  $\leq 0.5$  BCS were considered losing no to little BCS in early lactation, whereas animals losing  $> 0.5$  BCS were considered having substantial BCS loss.

### Statistical Analysis

Cow was considered the experimental unit in all analyses. Extreme data values ( $\geq 3$  SD above or below the mean) were manually evaluated before analyzing the data. Erroneous behavior data due to malfunctioning loggers were removed. Implausible measurements during farm visits were reviewed against the original paper forms, and erroneous data entries corrected.

Descriptive lameness incidence rate was estimated for the pre- and postpartum period (wk  $-8$  to wk  $12$ ), separately for primiparous and multiparous animals. Number of cow-weeks at risk was calculated using the exact method (Dohoo et al., 2009), assuming that lameness events occurred midway between visits. To evaluate when first lameness event occurred most frequently, the pre- and postpartum periods were split into 4-wk periods. Animals becoming lame in a previous period were no longer considered at risk. Cows with unknown calving date were excluded from incidence analyses, as were cows with  $< 3$  locomotion assessments (as the criteria for moderate lameness could not be met by these animals). Cows with  $< 2$  assessments after calving were excluded from postpartum incidence analysis. Unconditional (crude) sample odds ratio (**OR**) for becoming lame and for becoming severely lame after calving, depending on type of lesion found at trimming [grouped as no registered lesion, horn lesions (sole horn and white line lesions, and thin soles) or infectious lesions], was calculated from the raw data. No statistical

inference is provided for these descriptive analyses, as we did not have a priori hypotheses.

To evaluate the relationship between standing behavior and sole horn lesions, we used mixed-effects logistic regression models (binomial distribution, logit link function), with presence of sole hemorrhages or sole ulcers at trimming as the outcome. The models were fitted with maximum likelihood, using the `glmer` function in the `lme4` package (Bates et al., 2015). Separate models were fitted for the periods before, around, and after calving, and also for change in standing behavior from pre- to postpartum. Likelihood ratio tests were used to compare subsets of models, to assess if additional terms improved model fit. Herd was included in the models as a random intercept. Random slopes of average daily standing time and average daily longest standing bout were tested in the models, but were not retained as they did not improve model fit. Continuous independent variables, including standing behaviors, were centered at their means but not scaled. The distributions and relationships between independent variables were assessed graphically (package `GGally`; Schloerke et al., 2018). Pearson correlation coefficient was calculated for all linearly related continuous independent variables. Average daily longest standing bout and the longest standing bout 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 ( $r < 0.7$ ;  $n \geq 259$ ), and initially the behaviors were evaluated in the same models. This resulted in 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 2 standing behaviors. The same was found when analyzing change in average daily standing time and change in average longest standing bout, so separate models were fitted also for change in standing behavior.

Cow-level covariates included in the full models (based on the causal diagram) were parity (primiparous vs. multiparous), uncorrected milk production (kg) at 50 to 100 DIM, DIM at milk yield assessment, BCS at calving (BCS  $< 3.25$  vs. BCS  $\geq 3.25$ ), and BCS loss ( $\leq 0.5$  vs.  $> 0.5$  points) in early lactation. Not all animals were trimmed within the target period of 8 to 12 wk postpartum. To account for this, all cows were retained in the models and DIM at trimming was included as a covariate.

Biologically plausible 2-way interactions (standing behaviors  $\times$  BCS at calving, standing behaviors  $\times$  parity, parity  $\times$  BCS at calving, and parity  $\times$  BCS loss) were tested in separate models, as the full model did

not converge when interactions were included. Significant interactions were evaluated graphically. Separate models with parity  $\times$  BCS loss, and parity  $\times$  BCS at calving interaction terms did not converge due to insufficient data, and were evaluated by cross-tabulation. Complete data separation was present for parity  $\times$  BCS loss, as all multiparous cows with sole hemorrhages or sole ulcers at trimming lost  $\geq 1$  BCS in early lactation. Cross-tabulation did not indicate an interaction between parity  $\times$  BCS at calving. As interactions could not be tested in the full model (while controlling for the other independent variables), statistical inferences for these terms are not reported. Results from the final models include main effects only. Model fit was evaluated by assessing residuals at the higher (random effect) and lower (fixed effects) levels. Influential data points (high leverage or high discrepancy) for fixed effects were removed and models refitted to evaluate changes in model coefficients, as described in the Results section.

## RESULTS

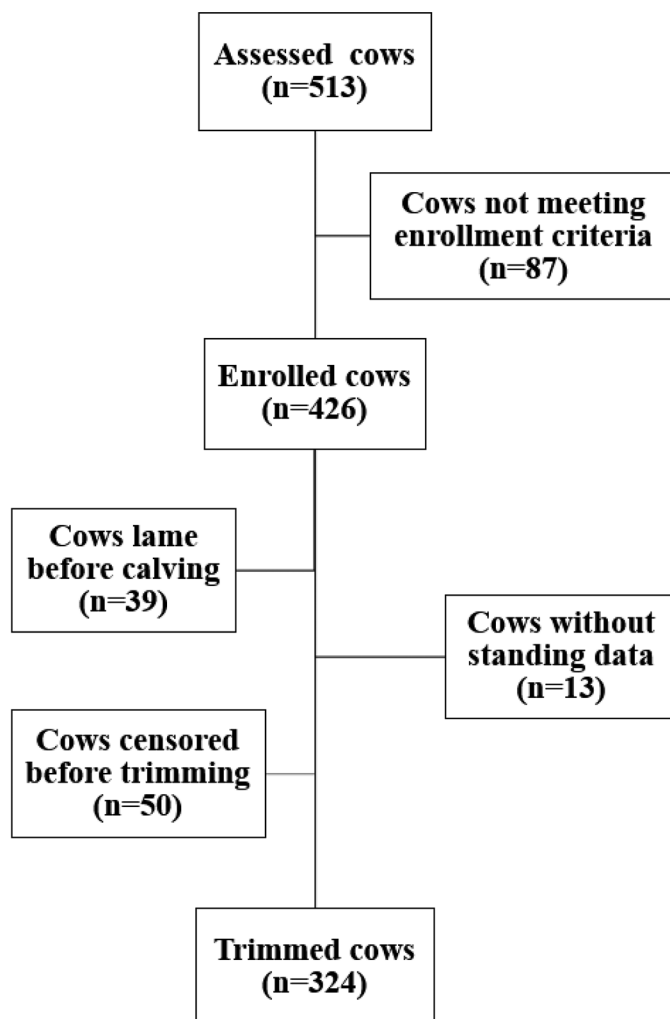
### Overview of the Data Set

A total of 513 animals were initially assessed for enrollment, and a median of 55.5 animals (range: 19 to 70) were enrolled per herd. See Figure 2 for the number of animals that were assessed, enrolled, and remained in the study until hoof trimming. Of the 87 animals that did not meet the enrollment criteria, 22 (25%), 25 (29%), and 38 (44%) were in parity 0, 1, and 2+ at first assessment, respectively; additional 2 cows had unknown parity but had calved previously. Of the enrolled animals, 13 completely lacked recorded standing behavior 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: 1 developed downer cow syndrome after calving, 6 either aborted or calved so early that standing behavior was not recorded, 1 had an unknown due date, 15 were culled, 16 were sold, 9 died on-farm (5 from unknown causes, 2 from mastitis, 1 from ketosis, and 1 from abomasal displacement), 1 could not be locomotion scored for  $> 2$  mo after calving as it was fitted with hobbles, and 1 was dangerous to handle. An additional 39 animals became lame before calving and were removed from the data set.

The number of animals in lactation number 0, 1, and 2+ was 189 (44%), 124 (29%), and 111 (26%) at enrollment (2 animals had unknown parity), whereas 149 (46%), 100 (31%), and 75 (23%) cows remained in the study until trimming, respectively. For trimmed animals, the median BCS at calving was 3.25 [interquartile



range (IQR): 3.00 to 3.75] for primiparous and 3.5 (3.25 to 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 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  $\geq 1$  BCS score in early lactation; the corresponding values for older cows were 17% and 72%, respectively. Mean  $\pm$  SD daily milk yield was  $31.7 \pm 5.9$  kg for first-calving animals, and  $43.7 \pm 7.9$  kg for multiparous cows; 3 animals lacked milk yield data.



**Figure 2.** The number of dairy cows assessed for, enrolled in, and remaining in the study until postpartum trimming. Animals with no previous registered severe horn lesion, and deemed sound 4 to 8 wk prepartum, were locomotion scored every other week from the dry period until trimming at  $11 \pm 2$  (range 6.4 to 19.1) wk postpartum. Cows becoming lame before calving, or lacking measures of standing behavior, were excluded from the study. Censored animals were removed from the herd based on decisions made by the herd manager.

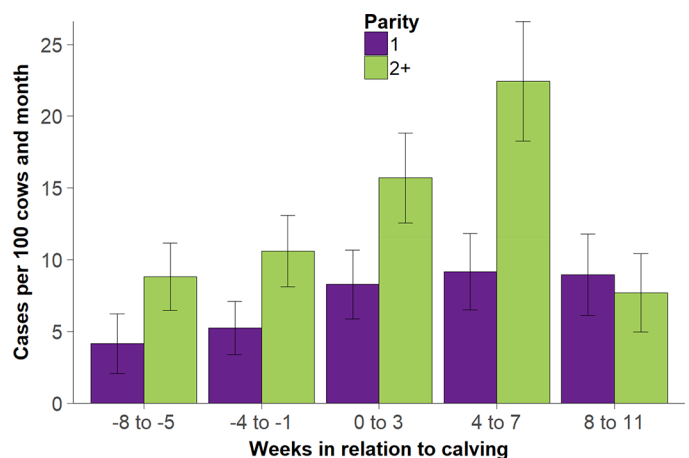
### Lameness Incidence

Enrolled cows were on average locomotion scored 10 times (IQR: 9 to 12) during the study. On 35 occasions we could not perform locomotion assessment on individual cows (0.8% of all assessments). On 2 occasions the cow was calving during the visit, on 12 occasions animals could not be found in their home pen, and during one visit the farmer did not allow assessment of the fresh cows because of high ambient temperature (resulting in 21 cows not being scored on that date). Thirty-one animals lacked 1 locomotion score, and 2 cows lacked 2 scores.

Figure 3 illustrates the lameness incidence from 8 wk before to 12 wk after calving, split in periods of 4 wk. The distribution differed between primiparous and multiparous cows, with older animals generally having higher incidence. Lameness incidence was lower during the prepartum period for both parity groups, compared with the postpartum period (primiparous: 4.8 vs. 8.7 cases/100 cows per month; multiparous: 9.3 vs. 15.6). The postpartum incidence of lameness remained stable at 8 to 9 cases/100 cows per month over the first 12 wk of lactation for the primiparous cows. Lameness incidence for multiparous cows was initially higher, but dropped to the level of primiparous cows 3 mo after calving.

### Hoof Lesion Prevalence

Of the enrolled animals that remained sound until calving, 324 were trimmed at  $11 \pm 2$  (mean  $\pm$  SD) wk



**Figure 3.** Lameness incidence during the prepartum period and early lactation, contrasting primiparous ( $n = 184$ ) and multiparous ( $n = 222$ ) cows monitored on 8 herds in the lower Fraser Valley region of British Columbia, Canada. Animals becoming lame in a previous period were no longer considered at risk. Lameness incidence was estimated using exact calculation, and error bars illustrate standard error [ $SE(p) = \sqrt{(A/t^2)}$ ,  $A$  = number of cases,  $t$  = time at risk].

postpartum. Sixty-two percent of these animals were trimmed within the target period 8 to 12 wk postpartum. Primiparous animals were trimmed at a median 79 (IQR: 69.0 to 91, range: 45 to 134) DIM, and older cows at 77 (IQR: 67.0 to 87.5, range: 48 to 128) DIM.

The hoof lesion prevalence was 4.3% for sole hemorrhages, 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 lesions, and the proportion of animals with lesions did not differ with parity (parity 1 = 23.5%; parity 2+ = 24.6%). Sole hemorrhages were found in 6.0% of primiparous ( $n = 149$ ) and 2.9% of multiparous ( $n = 175$ ) cows, whereas sole ulcers were found in 2.0% of primiparous and 5.1% of multiparous cows. The prevalence of infectious (0 to 56%) and horn (0 to 22%) lesions at postpartum trimming varied between farms.

### Postpartum Gait in Relation to Hoof Lesions

Compared with animals with no registered lesions at postpartum trimming, animals with horn lesions (sole horn lesions, white line lesions, and thin soles) had 2.5 times higher crude sample odds of becoming lame after calving, and 11.7 times higher crude sample odds of becoming severely lame. Of the 13 cows that had severe horn lesions (sole ulcers and white line disease) at trimming, 69% became lame, and 46% became severely lame between calving and trimming. Conversely, 28% of the 18 cows with milder horn lesions (slight separation in the white line, sole, and white line hemorrhages) at trimming became lame, and 11% became severely lame between calving and trimming. Animals with infectious hoof lesions had 3.2 times higher crude sample odds of becoming lame after calving and 5.0 times higher crude sample odds of becoming severely lame, compared with cows with no registered lesions.

Of the animals that became lame after calving, cows with no registered lesions ( $n = 61$ ) became lame at median 4.0 wk (IQR: 2.0 to 7.0) after calving. The corresponding values for animals with horn lesions ( $n = 14$ ) and infectious lesions ( $n = 24$ ) were 4.0 (IQR: 3.3 to 6.0) and 6.5 wk (IQR: 4.8 to 9.3), respectively. Seventy-six percent of the animals that became lame after calving were still lame in the end of the study period. Censoring occurred at  $12.6 \pm 3.0$ ,  $13.1 \pm 2.5$ , and  $13.0 \pm 2.3$  wk postpartum for lame animals with no, horn, and infectious lesions, respectively.

### Standing Behavior

Of the 324 animals with trim records, 316, 309, and 310 had standing behavior data before (d -14 to -2),

around (d -1 to 1), and after calving (d 2 to 14), respectively. Average daily standing time was normally distributed, with a mean  $\pm$  SD of  $12.1 \pm 1.6$ ,  $14.4 \pm 2.2$ , and  $13.8 \pm 1.7$  h/d for the 3 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 to 4.3; range: 1.7 to 12.1), 3.9 h/d (IQR: 3.1 to 4.8; range: 1.3 to 11.5), and 3.7 (IQR: 3.2 to 4.4; range: 1.5 to 11.7) h/d before, around, and after calving, respectively. The values for the longest standing bout of the period were 5.5 (IQR: 4.3 to 7.3; range: 2.4 to 21.7), 4.9 (IQR: 3.7 to 6.6; range: 2.1 to 18.1), and 5.9 (IQR: 4.8 to 7.3; range: 1.9 to 26.3) h, respectively.

Data were available both pre- and postpartum for 302 animals. The mean  $\pm$  SD change in average daily standing time was  $1.8 \pm 1.8$  h/d, whereas the corresponding value for change in average longest daily standing bout was  $0.1 \pm 1.3$  h/d (a positive value indicates increase in standing from before to after calving).

### Association Between Standing Behavior and Sole Hemorrhages or Sole Ulcers

For analyses of sole hemorrhages and sole ulcers, standing behavior data were available from 262, 253, and 256 animals before, around, and after calving, respectively, whereas 250 cows had data for change in standing behavior from before to after calving. There was no evidence for an association between standing behavior before or around calving and sole hemorrhages or sole ulcers (Table 4). After calving, an increase in average daily standing time (OR = 1.40, 95% CI: 1.07 to 1.86) and average longest standing bout (OR = 1.55, 95% CI: 1.14 to 2.16) were associated with increased odds of sole hemorrhages or sole ulcers (Table 4; Figure 4). Graphical evaluation of interactions (Figure 5), significant when tested in separate models during model construction, suggests that the relationship between daily standing time and sole hemorrhages or sole ulcers was stronger for multiparous cows. When removing the animal with most extreme postpartum average longest standing bout (a data point with high leverage), the  $P$ -value increased from  $P = 0.006$  to  $P = 0.057$ . As the findings for this animal conformed to the hypothesized higher risk of sole hemorrhages and sole ulcers for animals with extreme standing behaviors, it was retained in the final model. Random slope estimates for average daily standing time and average daily longest standing bout did not improve model fit, which indicates that the relationships between these standing behaviors and sole hemorrhages or sole ulcers at trimming were consistent in the enrolled herds. No evidence of an as-

**Table 4.** Results from multiple mixed-effects logistic regression models<sup>1</sup> used to evaluate the association between standing behaviors in late gestation and early lactation and the odds of sole hemorrhages or sole ulcers at 11 ± 2 wk postpartum in Holstein dairy cows (monitored in 8 herds in the lower Fraser Valley region of British Columbia, Canada)

Variable <sup>2</sup>	n <sub>tot</sub> (n <sub>sole</sub> ) <sup>3</sup>	Odds ratio (95% CI)	P-value
Average daily standing time (h/d)			
d -14 to -2	262 (25)	1.15 (0.87–1.55)	0.33
d -1 to 1	253 (22)	1.19 (0.94–1.51)	0.15
d 2 to 14	256 (24)	1.40 (1.07–1.86)	0.02
Average daily longest standing bout (h/d)			
d -14 to -2	262 (25)	1.08 (0.78–1.44)	0.62
d -1 to 1	253 (22)	1.07 (0.78–1.43)	0.67
d 2 to 14	256 (24)	1.55 (1.14–2.16)	0.006
Change in average daily standing time <sup>4</sup> (h/d)	250 (24)	1.24 (0.96–1.59)	0.09
Change in average daily longest standing bout <sup>4</sup> (h/d)	250 (24)	1.57 (1.09–2.28)	0.02

<sup>1</sup>Herd included as random intercept.

<sup>2</sup>A unit change in the different standing behaviors constitutes a 1-h increase in duration per day.

<sup>3</sup>Number of animals in total (n<sub>tot</sub>) and with sole hemorrhages or sole ulcers (n<sub>sole</sub>) differed between periods because of missing standing data.

<sup>4</sup>Positive value = standing behavior increased from the prepartum (d -14 to -2) to the postpartum (d 2 to 14) period.

sociation between increase in average daily standing time from before to after calving (OR = 1.24, 95% CI: 0.96 to 1.59) and sole hemorrhages or sole ulcers was found, due in part to one highly influential data point (Figure 4). This animal had the third highest average daily standing time before calving, which decreased to below the median value postpartum. Lameness did not affect postpartum behavior in this case, as the animal remained sound during the period standing behaviors were recorded. The cow was therefore retained in the final model. A 1 h increase in average daily longest standing bout raised the odds of sole hemorrhages or sole ulcers 1.57 times (95% CI: 1.09 to 2.28; Table 4).

The effects of the covariates included in the full models are reported in Table 5, and differed marginally between the models. No evidence was found that the main effects of parity and milk yield at 50 to 100 DIM were associated with sole horn lesions, but losing ≥1 BCS score was related to a higher risk of developing sole hemorrhages or sole ulcers in all models.

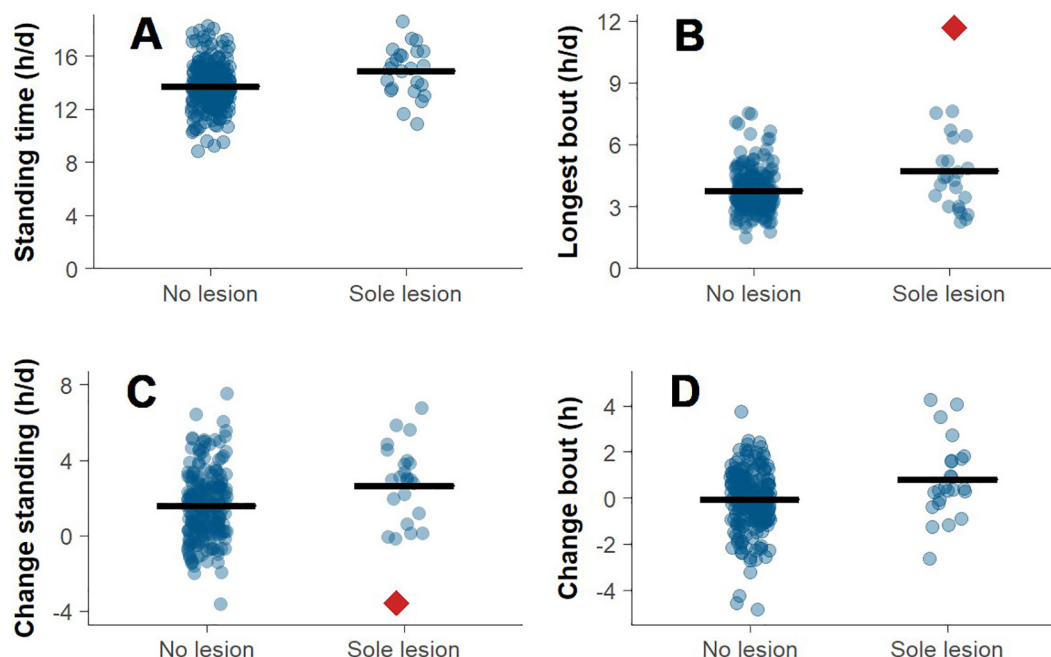
## DISCUSSION

The current study is the first observational study to evaluate the relationship between standing behavior during the transition period and the development of sole hemorrhages and sole ulcers in commercial herds. It is also the first to evaluate the relationship between the daily longest standing bout and sole hemorrhages or sole ulcers. The enrolled herds were typical of freestall farms in British Columbia, Canada, both regarding management practices and lameness prevalence (see von Keyserlingk et al., 2012, for comparison).

The indications of multicollinearity observed when testing average daily standing time and average longest standing bout duration in the same models suggest that both behaviors were associated with sole hemorrhages and sole ulcers through the same underlying mechanism. Animals with high average longest standing bout also had high average standing time. This finding suggests that it was difficult for cows to compensate for lost lying time due to long standing bouts on these commercial farms. This information should be considered when developing causal diagrams in future studies; we suggest that standing time should be viewed as an intervening variable to long standing bouts.

The relationship between standing behavior and sole hemorrhages and sole ulcers depended on period. We found no evidence of an association between sole hemorrhages or sole ulcers and standing behavior before and around calving, but daily standing time and longest bout duration after calving were related to sole hemorrhages or sole ulcers at postpartum trimming. Even though we did not observe a significant relation between standing behaviors before and around calving and sole hemorrhages or sole ulcers later in lactation, the direction of the association was the same as for postpartum standing behaviors. It is possible that increased standing prepartum also affected hoof health, but that our study was underpowered to detect this.

Based on initial interaction (average daily standing time × parity) models during model construction, the association between postpartum standing time and sole hemorrhages or sole ulcers might be stronger for multiparous cows. It is important to note that this interaction term could not be tested in the full model due to



**Figure 4.** Measured standing behaviors contrasting animals with no registered lesions and animals with sole hemorrhages or sole ulcers at trimming  $11 \pm 2$  wk postpartum. (A) Average daily standing time postpartum (2 to 14 DIM;  $n_{\text{tot}} = 256$ ,  $n_{\text{sole}} = 24$ ); (B) average daily longest standing bout postpartum (2 to 14 DIM;  $n_{\text{tot}} = 256$ ,  $n_{\text{sole}} = 24$ ); (C) change in average daily standing from before (d  $-14$  to  $-2$ ), to after (d 2 to 14) calving ( $n_{\text{tot}} = 250$ ,  $n_{\text{sole}} = 24$ ); and (D) change in average daily longest standing bout from before, to after calving ( $n_{\text{tot}} = 250$ ,  $n_{\text{sole}} = 24$ ), where  $n_{\text{tot}}$  = number of animals in total;  $n_{\text{sole}}$  = number of animals with sole hemorrhages or sole ulcers. Group means are illustrated with crossbars. Red diamonds illustrate the 2 most influential data points in mixed-effects logistic regression models with herd as random effect, used to evaluate the relationship between standing behaviors and sole horn lesions. Removal of the influential data point in graph B reduced the effect size and increased SE for average longest standing bout, which changed the  $P$ -value from 0.006 to 0.057. 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 0.09 to 0.03.

low power, so strong inferences should not be drawn from this finding. Animals that increased duration of the longest daily standing bout from before to after calving had an increased risk of developing sole hemorrhages or sole ulcers; this result is consistent with the idea that an increase in the duration of mechanical load may be damaging. Our descriptive results for standing behavior show that the duration of the average longest standing bout varied considerably between cows, and that some cows have very long individual standing bouts (up to 26 h). In a case-control study, Sepúlveda-Varas et al. (2018) evaluated the relationship between lying behavior during the transition period and horn lesions at 8 to 16 wk postpartum in grazing primiparous cows. Similar to our findings, this study found that cows that developed horn lesions (85% of the cows had sole hemorrhages or sole ulcers, 15% had white line disease) in peak to mid lactation spent less time lying (i.e., more time standing) in the weeks after calving.

Newsome et al. (2017b) found that animals with little soft tissue between the sole and the pedal bone were more likely to develop horn lesions, and that the sole soft tissue was the thinnest the first week postpartum. Their findings suggest that excessive mechanical load

may be particularly damaging to hoof health immediately following calving, potentially explaining why only postpartum standing behaviors were associated with sole hemorrhages and sole ulcers in our study. Previous research (Chapinal et al., 2009; Proudfoot et al., 2010) reported that cows that developed sole hemorrhages or sole ulcers during mid lactation spent more time standing in the weeks before calving. The pen size used for lactating cows in these studies was small (12 to 20 cows per pen) compared with the commercial herds enrolled in the current study. It is possible that shorter time outside the pen for milking could explain why no relationship was found between postpartum standing behavior and sole hemorrhages and sole ulcers in the previous studies (see below for a more comprehensive discussion about milking duration).

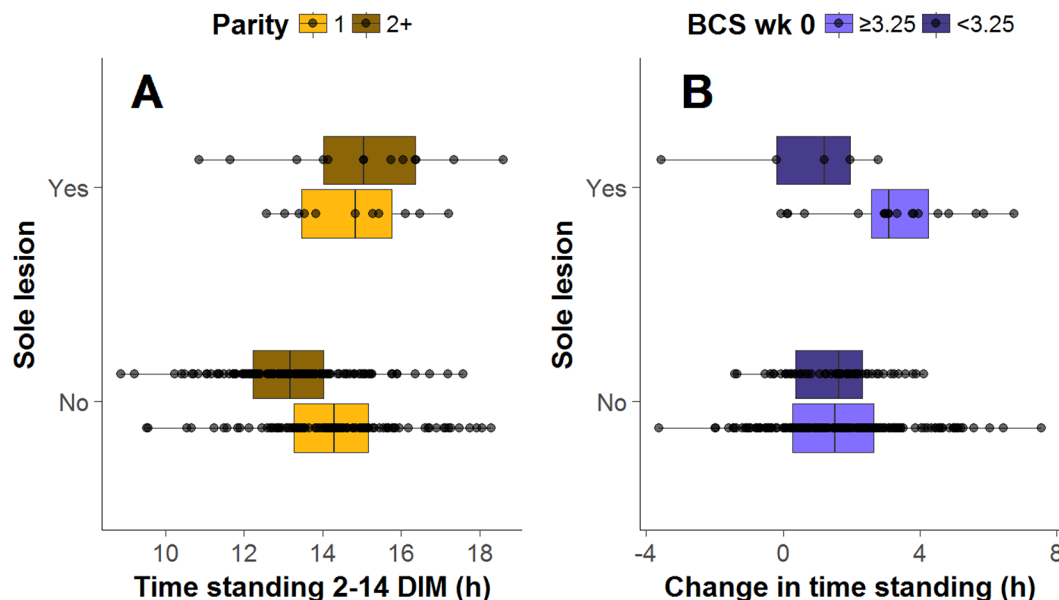
Ito et al. (2009) measured daily lying time over 5 d in 2,033 high-producing cows from 43 freestall herds. The authors reported a wide range in average daily lying time at the cow-level, corresponding to 4.5 to 19.8 h/d average daily standing time over the 5-d period. Proudfoot et al. (2010) evaluated transition cow standing behavior relative to trim findings later in lactation. Cows that developed sole hemorrhages or sole ulcers

spent on average 13.9 and 15.6 h/d standing during the last 2 wk before and 24 h after calving, respectively. Omontese et al. (2020) graphically presented predicted daily lying time between 18 and 116 DIM, contrasting cows with no lesions and cows with hoof lesions at 120 DIM (88% of hoof lesions were sole hemorrhages). At 18 DIM, the predicted daily lying time for cows that later developed hoof lesions was approximately 10 h/d (corresponding to a standing time of 14 h/d), while predicted lying time for cows that did not develop hoof lesions was approximately 10.5 h/d (standing 13.5 h/d). The measured average daily standing time in our study ( $12.1 \pm 1.6$ ,  $14.4 \pm 2.2$ , and  $13.8 \pm 1.7$  h/d before, around, and after calving, respectively) was similar to the 2 latter studies, and cow-level average daily standing time postpartum (range 8.9 to 18.6 h/d) fell within the range reported by Ito et al. (2009).

Parlor-milked cows spend a considerable portion of their day outside of the home pen, with pen-wise milking times ranging between 0.7 to 8.0 h/d in a Canadian study comprising 111 farms (Charlton et al., 2014). As dairy cows enter the parlor in a nonrandom order (Cook and Nordlund, 2009), some animals will consistently have a longer return time to the home pen. This variability in return time is likely one reason why postpartum duration of daily longest standing bout differed between the cows in our study (illustrated by the

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 for 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 postpartum and sole hemorrhages or sole ulcers in our study, we propose that animals with long return times from milking are more likely to develop sole horn lesions, and suggest that further work evaluate this hypothesis.

The frequent locomotion assessment in our study revealed distinct time patterns for animals becoming lame after calving, depending on the type of lesion recorded at trimming. For cows with no registered lesions versus those with horn lesions, the times from calving to lameness onset were similar. The majority of lame animals without lesions at trimming (47 of 61 cows) had impaired gait for  $\geq 6$  wk before leaving the study, making it unlikely that they were misclassified as lame. Researchers were not present at trimming, but lesions were assessed by professional trimmers in accordance with the field guide used by certified trimmers in Western Canada. Mild hemorrhages (lesions corresponding to severity 1, as described by Leach et



**Figure 5.** Graphical presentation of significant interactions tested in separate mixed-effects logistic regression models analyzing the relationship between sole hemorrhages or sole ulcers and standing behaviors. The full models did not converge when the interactions were included. Standing behavior was summarized per animal into 3 periods: before (d -14 to -2), around (d -1 to 1), and after (d 2 to 14) calving. (A) Interaction between parity and average daily standing time after calving ( $n_{\text{tot}} = 256$ ,  $n_{\text{sole}} = 24$ ); (B) interaction between BCS at calving and change in average daily standing time from before to after calving ( $n_{\text{tot}} = 250$ ,  $n_{\text{sole}} = 24$ ).  $n_{\text{tot}}$  = number of animals in total;  $n_{\text{sole}}$  = number of animals with sole hemorrhages or sole ulcers. The center line indicates the median value, the box the interquartile range, and the whiskers the range. Dots illustrate individual data values.



al., 1998) were not registered, which likely explains why the prevalence of sole hemorrhages in the current study (4.3%) was substantially lower than what has been reported in previous research (55%; Capion et al., 2009; 68%; Randall et al., 2016). It is possible that some of the lame animals with no trim findings were lame due to sole hemorrhages that appeared mild and were thus not registered at trimming, although sole hemorrhages are not generally related to impaired gait (Flower and Weary, 2006; Chapinal et al., 2009; Tadich et al., 2010). Alternatively, some of these animals may have been lame due to painful conditions in the leg. In our study, animals with horn lesions were most likely to become severely lame after calving, adding to the notion that these hoof lesions negatively affect cow welfare.

Multiple studies have shown that horn lesions often reoccur (Hirst et al., 2002; Machado et al., 2011), so

previous horn lesions could have influenced the association between standing behaviors and sole hemorrhages or sole ulcers in our study. To reduce this risk, we excluded animals with known history of severe horn lesions based on the herds' trim records. Although this filter was likely too coarse to allow for identification of all animals with severe lesions, the exclusion of cows with impaired hoof health may explain why we did not find evidence of an association between parity and the risk of developing sole hemorrhages or sole ulcers [the univariate association between parity and sole hemorrhages or sole ulcers was nonsignificant for all periods ( $P > 0.8$ ); data not shown]. This finding is in contrast to earlier research (e.g., Amory et al., 2008; Newsome et al., 2017a), and supports the suggestion made by Randall et al. (2018) that the effect of age on horn lesion risk is mediated through long-term pathological changes

**Table 5.** Covariates included in the mixed-effects logistic regression models<sup>1</sup> reported in Table 4<sup>2</sup>

Covariate	Odds ratio (95% CI)			
	Before <sup>3</sup>	Around <sup>3</sup>	After <sup>3</sup>	Change <sup>3</sup>
<b>Standing time models</b>				
Intercept	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.0 (0.0–0.1)
BCS at calving			Referent	
≥3.25				
<3.25	1.8 (0.5–6.1)	2.3 (0.6–8.1)	2.0 (0.6–6.4)	2.1 (0.6–6.8)
BCS loss			Referent	
<1.0				
≥1.0	6.8 (1.7–47.6)	6.7 (1.5–47.7)	7.2 (1.8–49.9)	8.1 (1.9–57.3)
Milk yield (kg)	1.1 (1.0–1.1)	1.0 (1.0–1.1)	1.1 (1.0–1.1)	1.0 (1.0–1.1)
DIM (milk yield) <sup>4</sup>	1.0 (1.0–1.0)	1.0 (0.9–1.0)	1.0 (1.0–1.0)	1.0 (1.0–1.1)
Parity			Referent	
1				
2+	0.5 (0.1–1.8)	0.6 (0.2–2.5)	0.7 (0.2–2.5)	0.6 (0.2–2.2)
DIM at trimming	1.0 (1.0–1.1)	1.0 (1.0–1.1)	1.0 (1.0–1.1)	1.0 (1.0–1.1)
Variance RI <sup>4</sup>	0.49	0.50	0.10	0.03
<b>Standing bout models</b>				
Intercept	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.0 (0.0–0.1)	0.0 (0.0–0.1)
BCS at calving			Referent	
≥3.25				
<3.25	1.9 (0.5–6.1)	2.2 (0.6–7.7)	2.2 (0.6–7.3)	2.4 (0.7–7.9)
BCS loss			Referent	
<1.0				
≥1.0	7.1 (1.7–49.3)	6.4 (1.5–45.4)	6.8 (1.7–47.4)	8.6 (2.0–60.5)
Milk yield (kg)	1.1 (1.0–1.1)	1.0 (1.0–1.1)	1.1 (1.0–1.1)	1.0 (1.0–1.1)
DIM (milk yield) <sup>4</sup>	1.0 (1.0–1.0)	1.0 (0.9–1.0)	1.0 (1.0–1.1)	1.0 (1.0–1.1)
Parity			Referent	
1				
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)
Variance RI <sup>5</sup>	0.37	0.48	0.06	0.00

<sup>1</sup>Herd included as random intercept.

<sup>2</sup>The reported estimates were obtained from the full models and so reflect the direct effect rather than the total effect of the covariates on the outcome.

<sup>3</sup>Period definitions are in relation to calving; before = d –14 to –2 ( $n_{\text{tot}} = 262$ ,  $n_{\text{sole}} = 25$ ); around = d –1 to 1 ( $n_{\text{tot}} = 253$ ,  $n_{\text{sole}} = 22$ ); after = d 2 to 14 ( $n_{\text{tot}} = 256$ ,  $n_{\text{sole}} = 24$ ); change = change in standing behavior from before to after calving ( $n_{\text{tot}} = 250$ ,  $n_{\text{sole}} = 24$ ).  $n_{\text{tot}}$  = number of animals in total;  $n_{\text{sole}}$  = number of animals with sole hemorrhages or sole ulcers.

<sup>4</sup>DIM when 24-h uncorrected milk production in kilograms was recorded.

<sup>5</sup>RI = random intercept.

in the inner structures of the hoof. Excluding cows with impaired hoof health resulted in a high proportion of first parity animals (44%) in the current study. When evaluating the association between lying behavior and horn lesions in pasture-based systems, Sepúlveda-Varas et al. (2018) included only primiparous cows in their study. Thus, the results of this study and the current study both indicate that increased standing time can cause mechanical damage to the hooves in animals with previously good hoof health.

Although both parity groups were equally likely to develop sole horn lesions, multiparous animals more often were diagnosed with sole ulcers. This finding has been reported in earlier studies (Manske et al., 2002; 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 hemorrhages generally occur earlier than sole ulcers after calving (e.g., Newsome et al., 2017a). However, the disparity in lesion severity between parities could not be explained by differences in trim date relative to calving in the current study. It is possible that the more severe lesions found in multiparous cows could be a result of osteophyte formation on the pedal bone. When present, these protruding bone formations likely creates pressure points on the sole's horn producing cells (the corium; Newsome et al., 2016). As the size of osteophytes on the pedal bone generally increase with age (Tsuka et al., 2012; Newsome et al., 2016), prolonged standing could result in more severe injuries in 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 within the hoof capsule had not yet developed in these animals. Although animals with sole hemorrhages were less likely to be lame in the current study, it has been suggested that inflammation caused by the initial trauma may exaggerate osteophyte formation on the pedal bone, 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 hemorrhages and small sole ulcers 2 to 4 mo postpartum (the animals were grouped together for analysis, 89% had hemorrhages) had a higher risk of lameness in future lactations.

Our primary aim was to obtain unbiased estimates for the effects of standing behaviors on the risk of developing sole hemorrhages and sole ulcers, so potential confounders described in our causal diagram were included as covariates in the full models regardless of their univariate relationship with the outcome. Postpartum lameness was considered an intervening variable for standing behaviors and was hence not included in the

models. As potential confounders for the different covariates were not included in the models, the covariate estimates may be biased (see Westreich and Greenland, 2013) and represent only the direct effect of the covariates on the outcome (arrows going directly from the covariates to the outcome in the causal diagram). The presented covariate estimates should be interpreted in the context of causal modeling.

The low number of animals with recorded sole hemorrhages and sole ulcers made the current study underpowered, and the interactions suggested in our causal diagram (e.g., parity  $\times$  BCS loss, and parity  $\times$  BCS at calving) could not be statistically tested in the full models. It is questionable if significant (or non-significant) results for tested interactions are robust, considering that some cell counts were very low. Many risk factors for sole hemorrhages and sole ulcers are interrelated, and could potentially influence the effect of other independent variables. Currently our understanding of how different risk factors interact is incomplete, and additional large-scale studies are needed to better understand the etiopathogenesis of sole horn lesions.

## CONCLUSIONS

This study evaluated how standing behavior during the transition period relates to sole hemorrhages and sole ulcers in peak lactation in commercial dairy herds. Increased time spent standing and long standing bouts postpartum were associated with an increased risk of sole hemorrhages or sole ulcers. Cows that developed sole ulcers were more likely to become severely lame after calving, compared with animals with no registered lesions at trimming.

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