Associations of herd- and cow-level factors, cow lying behavior, and risk of elevated somatic cell count in free-stall housed lactating dairy cows

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ABSTRACT
Recent evidence suggests that the risk of intramammary infection in dairy cows is related to lying patterns. The objectives of this study were to quantify the standing and lying behavior of dairy cows milked 3×/d, determine the cow- and herd-level factors associated with these behaviors, and relate these findings to the risk of an elevated somatic cell count (SCC). Five commercial free-stall dairy herds in Eastern Ontario, milking 3×/d, were enrolled in a longitudinal study. Forty Holstein-Friesian cows/herd were randomly selected as focal animals based on days in milk (<200 d) and SCC (<100,000 cells/mL). Farms were followed for 4, 5-week periods. Individual-cow SCC was recorded at the beginning of each period and end of the final period. Elevated SCC (eSCC) was used as an indicator of subclinical mastitis. A new incident eSCC was defined as an individual cow that started the period with a SCC <100,000 cells/mL but whose next SCC exceeded 200,000 cells/mL. Lying behavior was recorded 5 d after each milk sampling using data loggers. For these 5 d, individual milking times and feeding times were also recorded. On d 1 of each recording period 2 trained observers scored focal cows for hygiene and lameness. Throughout the course of the study, cows averaged 11.2 h/d of lying time, split into 8.6 lying bouts/d that were on average 84.6 min in length. Later lactation cows had longer daily lying times that were split into fewer lying bouts of longer duration than cows earlier in lactation. Lame cows had longer daily lying times and lying bout durations than non-lame cows. Cows with greater milk yield had lower lying times than lower producing cows. Average post-milking standing time across the study herds was 103 min. Manipulation of feed (feed delivery or push-up) by the stockperson, in the hour before milking or shortly thereafter, resulted in the longest post-milking standing times. Over the study period, 48 new eSCC were detected, resulting in a mean herd incidence rate of 0.91 eSCC/cow-year at risk for all study herds. A non-linear relationship between post-milking standing time and eSCC incidence was found; compared to those cows that lie down <90 min after milking, cows that lie down for the first time >90 min after milking had a lower risk of acquiring a new eSCC. The risk of experiencing an eSCC was also increased in multiparous cows, and in those cows with a higher SCC at the beginning of the study. These results indicate that management practices that promote post-milking standing time, such as the manipulation of feed delivery around milking times, should be encouraged to reduce the risk of cows experiencing new eSCC.

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1. Introduction

Mastitis is the most economically significant disease impacting the global dairy industry (Seegers et al., 2003). Given the complexity of the disease, complete elimination of intramammary infection is not feasible at this time (Smith, 1983). Thus, understanding farm management practices that reduce the incidence of mastitis is essential, from both an economic and animal welfare standpoint (Barkema et al., 1999).

A long-accepted management practice assumed to reduce the risk of mastitis is to promote longer standing times after milking. The presence of feed, particularly fresh feed, has been shown to encourage longer post-milking standing times (Tyler et al., 1997; DeVries and von Keyserlingk, 2005; DeVries et al., 2010). Keeping cows on their feet for a certain period of time after milking will increase the likelihood that the teat canals have closed prior to the udder contacting the stall surface when the cow lies down, thus decreasing the odds of bacterial penetration of the teat (Tyler et al., 1997; Johansson et al., 1999). A study of tie-stall housed cows observed that cows that laid down, on average between 40 and 60 min after milking, tended to have a decreased risk of acquiring a new environmental intramammary infection (IMI) compared to those cows that laid down within 40 min after milking (DeVries et al., 2010).

In the study by DeVries et al. (2010), the mean post-milking standing duration of the studied tie-stall housed cows was 79 min. This is much longer than that previously reported for free-stall housed cows (milked 2×/d), ranging from 33 to 62 min (Tyler et al., 1997; DeVries and von Keyserlingk, 2005; DeVries et al., 2005; Fregonesi et al., 2007). These results provide some evidence that in free-stall housing systems, a large percentage of cows lie down relatively quickly (within 40 min) after returning from milking. One possible explanation for this may be the additional time required for these cows to access feed in addition to the time spent standing before and during milking. In a study of 16 free-stall farms, it was found that increased time spent milking was associated with a decrease in time spent feeding and lying (Gomez and Cook, 2010). Further, lying behavior has been shown to increase during the night and early morning hours, unless disrupted by management practices (Mattachini et al., 2011). Decreased post-milking standing durations observed in free-stall housed, parlor milked, cows may thus be a consequence of these animals attempting to regain lost lying times. One management practice that could further exacerbate this behavior is milking frequency. In herds with 3×/d milking, cows are being milked on average every 8 h: a practice that arguably disrupts their diurnal behavior patterns. Therefore, it is expected that the tendency for free-stall housed cows to lie down soon after milking may be further exacerbated when cows are milked more frequently.

There were three objectives for this study: (1) to quantify the standing and lying behavior, with particular emphasis on post-milking standing time, of dairy cows milked 3×/d, (2) to determine the cow- and herd-level factors associated with lying behavior, and (3) to relate these findings to the risk of experiencing an elevation in somatic cell count (SCC). It was hypothesized that post-milking standing times would be shorter when fresh feed was not offered near the time of milking, and that those cows which lie soonest after milking would have a higher risk of acquiring a new elevated SCC (eSCC).

2. Materials and methods

2.1. Farm selection

Five commercial dairy farms in Eastern Ontario, Canada were recruited for participation in this study. Herds were selected as a convenience sample. Selection was based on the criteria that they had free-stall housing, milked cows in a parlor, milked 3×/d, participated in a dairy herd improvement (DHI) program, were predominantly Holstein-Friesian (>90%), and had >120 lactating cows in the herd. Overall, these farms had a mean herd size of 337 (range: 144–566) lactating cows and a mean adjusted 305 d milk yield of 10,097 kg (range: 9541–10,699 kg). The mean annual bulk milk SCC was 247,000 cells/mL (range: 141,000–316,000 cells/mL). The study was conducted on these herds between September 2011 and February 2012. The farms were on a rolling start date corresponding to their DHI test dates.

2.2. Animal selection

Forty focal cows per herd were randomly selected from all herds. Eligible cows were <200 d days in milk (DIM), ensuring that they would be available for 2 further DHI tests prior to dry-off, and had a SCC <100,000 cells/mL at the most recent DHI test. Lactation number and DIM were recorded from farm records for each focal cow and validated using DHI data.

2.3. Milk sampling and analysis

Cow-level SCC testing was conducted using composite milk samples taken approximately every 35 days, as part of the DHI program, and on-line reports of results were made available a few days after testing. Farms were enrolled in the study immediately following a regularly scheduled DHI test date, with each subsequent period beginning after the next DHI test date (which occurred on average at 5-wk intervals; average interval was 37 ± 13 d (mean ± SD)). A total of 4 observation periods were targeted on each farm. Milk sampling was conducted by the DHI customer service representative assigned to the farm and milk samples shipped overnight to the CanWest DHI Laboratory (Guelph, ON, Canada), and were analyzed for SCC using the Fossomatic method (Fossomatic 5000 series, Foss Electric, Hillerød, Denmark).

Somatic cell counts (eSCC) were used as a proxy for subclinical mastitis (Dufour et al., 2011 when >200,000 cells/mL). A new case was identified as one where the previous test was <100,000 cells/mL and the most recent test was >200,000 cells/mL (Dohoo and Leslie, 1991; Schepers et al., 1997; Schukken et al., 2003). Thus, over the 4, 5-week periods, the maximum number of new eSCC
any individual cow could develop was 2. Incidence rate of eSCC was calculated as: number of new eSCC/# of cows at risk/d at risk × 365 d/year (Dohoo et al., 2009). To determine required number of cows and observation periods, a power analysis was conducted a priori using PASS software (PASS, Kaysville, UT, USA). For the calculation, beta was set to 0.20 (80% power), and alpha was set to 0.10. Estimates for incidence rates of infection, as well as for variability in observation of standing and lying behavior, were taken from previous findings (DeVries et al., 2010, 2011a).

2.4. Standing and lying behavior

Lying behaviors were recorded using data loggers (HOBO Pendant C Data Logger, Onset Computer Corporation, Pocasset, MA, USA) that record leg orientation in 1-min intervals (Ledgerwood et al., 2010). Data loggers were placed on the hind limb of focal cows with veterinary bandaging tape (Vetrap Bandaging Tape, 3 M, London, ON, Canada) for the first 5 d of each period following DHI testing. Ito et al. (2009) demonstrated that recording lying behavior for 3 d produced an accurate estimate of herd-level lying behavior using the same technology as utilized in this study. Thus, a 5 d recording period was chosen to improve the accuracy of the observations. Recordings made in the first 5 d following the DHI test were assumed to reflect a cow’s normal (typical) behavior and cows were identified as infection free when SCC <100,000 (Medrano-Galarza et al., 2012). A cow’s typical lying behavior (when not infected) was assumed to be related to her risk of acquiring a new infection based on the SCC obtained at the next DHI test. Feed delivery and feed push-up were recorded by the herd manager on the same days as lying behavior. The interval between feed delivery and milking time was calculated for each cow.

Standing and lying times (min/d), lying bout duration (min/bout), and lying bout frequency (#/d) were calculated, as per description by Ledgerwood et al. (2010), using the recorded data. Pre-milking standing duration (min) was defined as the interval between the last observation of lying prior to milking and the termination of milking. Post-milking standing duration (min) was defined as the interval between the termination of milking and the initial observation of lying following milking.

2.5. Cow-based measures

Individual milking times and milk production were obtained from parlor records obtained at each farm. Focal cows were scored for cleanliness in the milk parlor using a 4-point scale (1 = very clean to 4 = very dirty) hygiene scoring system (www.vetmed.wisc.edu/dms/fapm/fapmtools/4hygiene/hygiene.pdf; Cook and Reinemann, 2007) on the first day of each 5 d recording period. The udder, lower legs, and upper legs-flank were evaluated separately. locomotion was scored as cows exited the parlor on the first day of the 5 d recording period using a 5-point scale (1 = normal to 5 = severely lame; Flower and Weary, 2006). For statistical analysis, lameness was dichotomized into lame (gait score = ≥3) and not lame (gait score = <3). Both scoring procedures were completed by the same two trained observers on all farms for all periods and their scores were averaged for each cow. For both hygiene and locomotion scoring, training occurred by providing the two observers the scoring scales, along with visual examples (photographs for hygiene and video for lameness) for each score to study and practice with. The observers practiced scoring individual cows (n = 40) before the start of the study; inter-observer reliability of this practice scoring was tested and correlated to acceptable standards (r > 0.75).

2.6. Herd, housing, and management data

A questionnaire, adapted from Dufour et al. (2010, 2011), was administered on each farm before the commencement of the study. Farm owners or herd managers were questioned regarding housing and management practices, including milking practices, stall dimensions, and cleaning. Although all farms fed a TMR, bedding type and frequency of delivery, feeding regimen (both frequency of feed delivery and feed push-ups), and cleaning systems varied by farm. Feeding frequency was either 1 × d/n (n = 3) or 2 × d/n (n = 2). Feed push-ups ranged in frequency from 0 to 5 × d. Mean free-stall stocking density (# cows/stall) for all 5 farms was 104% (range: 95–114%). Linear bunk space per cow averaged 0.66 m/cow (range: 0.36–1.22 m). The median parlor capacity was 24 (range: 12–36) cows. While daily milking times were variable by farms, each farm did maintain an 8 h interval between milking start times. Only one farm used sand bedding while the other 4 used mattresses or waterbeds partially covered with either straw or composted manure solids. The sand bedded farm used deep bedding (>2 cm) while two of the mattress based farms provided bedding (<2 cm), and the other farms used >2 cm of bedding. Four of the farms docked tails and 3 clipped or flamed udders. Two farms had scheduled gait scoring; one farm recorded gait scores 1 ×/year while the second recorded 2 ×/year, a third farm just observed cows for lameness at milking time, and the other two farms had no specific gait scoring practices. All farms cleaned stalls 3 ×/d, corresponding to milking times, while alleyways were cleaned either by automatic scrapers (3 farms) or tractors (2 farms), with cleaning schedules ranging from 2 ×/week to 24 ×/d. Cows entered a holding pen prior to milking and farms milked by group according to production and/or parity. Focal animals were randomly selected to ensure representation across all of the farms’ milking groups. All farms engaged in pre- and post-milking cleaning procedures employing teat dips or foams pre- and post-milking; 2 farms utilized foam in their pre-milking cleaning procedure while all farms used a teat dip post-milking. Milking practices on all farms ensured that all teats were cleaned and dried before applying the milking machines that had automatic take offs. Two of the farms encouraged post-milking standing by pushing-up feed at the time cows returned from milking while the other 3 farms had no specific practices to encourage post-milking standing. Any variables that were unique to a specific herd were not retained as an explanatory variable in the statistical analyses since its measure of association
would be perfectly confounded by other characteristics specific to that herd.

2.7. Statistical analysis

Prior to analyses, all data were screened for normality using the UNIVARIATE procedure of SAS (SAS Institute Inc., 2009). Cow SC Cs at the study beginning were slightly right skewed and, thus, transformed by the natural logarithm. All cow-level data were summarized across each of the 5-d observation periods for each cow. The associations between dependent behavioral variables (lying time, lying bout frequency and duration, post-milking and pre-milking standing time) and possible cow-level and herd-level explanatory (independent) fixed-effect variables were analyzed with multivariable linear mixed models using the MIXED procedure of SAS (SAS Institute Inc., 2009), treating period as a repeated measure. The models included random effects from period and week within period. Cow within period was included in the models as the subject of the repeated statement. Initially, unconditional associations between independent variables and dependent variables were examined. Only independent variables with $P \leq 0.10$ in this initial screening were included in multivariable linear regression models. The CORR procedure of SAS was used to check for correlations between the retained explanatory variables. If two variables were highly correlated ($r > 0.8$), the one with the lower $P$ value in the unconditional associations was retained. For the multivariable models, effects were considered significant at $P \leq 0.1$. Manual backward elimination of non-significant effects was used to construct the final multivariable models. Period was not retained in any of the final models, thus no estimates for this effect are further presented. For the resultant models, plausible 2-way interactions were examined and retained if $P \leq 0.10$. Only those results retained in the final multivariable models are further presented. The covariance structure was first-order autoregressive or compound symmetry depending upon best fit according to Schwarz’s Bayesian information criterion. The association of the various cow-level and farm-level explanatory variables on the occurrence or non-occurrence of an eSCC was assessed using a random intercept mixed logistic model using the GLIMMIX procedure (distribution = binomial and link = logit) of SAS (SAS Institute Inc., 2009). As new eSCCs were assessed at the cow level, both herd and cow within herd were considered random. Unconditional associations were estimated in the described model to screen all potential explanatory variables. For variables measured on a continuous scale, linearity of the relationship between the variable and the occurrence of an eSCC was assessed by categorizing the continuous variable and visual inspection of plots of the odds ratio against mean values of the categories. Variables with $P \leq 0.20$ were retained for model building. The CORR procedure of SAS was used to check for correlations between the kept explanatory variables. If two variables were highly correlated ($r > 0.8$), the one with the lower $P$ value in the unconditional associations was retained. These kept variables were then included in a multivariable analysis using the above mentioned model. Effects in the multivariable logistic model were considered significant at $P \leq 0.1$. Manual backward elimination of non-significant effects was used to construct the final multivariable model. From the resultant model, plausible 2-way interactions were examined and retained if $P \leq 0.10$. Only those results retained in the final multivariable logistic model are further presented.

One of the farms was removed from the study after period 1 due to switching from parlor milking to an automated milking system; when this farm was enrolled in the study they had indicated they would not be making the switch between the 2 milking systems until a later date however unforeseen circumstances pushed this date forward. Two farms completed only 3 periods due to one farm switching from 3×/d to 2×/d milking due to a staff shortage and the final DHI test for the second farm failed to include SCC data. One cow on one farm was sold before the end of the first period, thus 199 cows completed the first period. Throughout the remainder of the study various cows were removed from the study as they were either culled or dried off. A complete data set (for 4 periods) was obtained for 67 cows from 2 of the 5 farms, however, all cow data were used in the analysis regardless of whether a cow had a complete data set.

### Table 1
Descriptive summary of the cows sampled within each study herd at the beginning of the study period.

<table>
<thead>
<tr>
<th>Herd</th>
<th>Cows sampled/ herd</th>
<th># of periods</th>
<th>Mean parity</th>
<th>Mean DIM (x1000 cells/mL)</th>
<th>Mean beginning SCC</th>
<th>Mean 305 d milk production (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>3a</td>
<td>2.2</td>
<td>115</td>
<td>51</td>
<td>9845</td>
</tr>
<tr>
<td>2</td>
<td>39b</td>
<td>3c</td>
<td>2.1</td>
<td>73</td>
<td>41</td>
<td>9820</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>4</td>
<td>2.2</td>
<td>68</td>
<td>36</td>
<td>10,777</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>1d</td>
<td>2.0</td>
<td>79</td>
<td>34</td>
<td>10,250</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>4</td>
<td>2.5</td>
<td>66</td>
<td>33</td>
<td>11,845</td>
</tr>
<tr>
<td>Total</td>
<td>199</td>
<td>–</td>
<td>2.2</td>
<td>74</td>
<td>39</td>
<td>10,521</td>
</tr>
</tbody>
</table>

a This herd was switched to 2×/d milking before the completion of period 4.

b One of the focal animals was sold before the completion of period 1.

c The final DHI test for this herd failed to include SCC data for the end of period 4.

d Herd switched to robotic milking before the completion of periods 2.

### 3. Results

Parity, DIM, initial SCC, and milk production of the study cows were similar across study herds (Table 1). Due to low selection availability on one farm (i.e. the farm lacked 40 cows with baseline SCC <100,000 cells/mL), one of the
40 enrolled cows had a baseline SCC of 125,000 cells/mL. Low selection availability on another farm required selection of a cow at 203 DIM. Across the study, a total of 669 observations of lameness and hygiene scores were made. The average, SD, and range values for these measures, as well as for standing and lying behavior, across all cows are found in Table 2.

3.1. Factors associated with lying behavior

Lameness was associated with both lying time and lying bout duration (Table 3). Lame cows were more likely to have longer daily lying times and lying bout durations than non-lame cows. Daily milk yield was negatively associated with lying time, with cows of higher milk production having shorter daily lying times; those cows above the study average daily milk yield (36.8 kg/d) had an average daily lying time of 10.6 h/d compared to 11.9 h/d for those cows below the average daily milk yield. Days in milk were associated with lying time, lying bout frequency, and lying bout duration. Cows with higher DIM were more likely to have longer lying times, fewer lying bouts, and longer lying bouts than cows earlier in their lactation. Parity was associated with lying bout frequency; cows of greater parity had fewer lying bouts per day.

3.2. Factors associated with pre- and post-milking standing time

Feed manipulation (feed delivery or push-up) occurred on average 62 min before milking (range: 867 min before milking to 432 min after milking). The relationship between duration of post-milking standing time and interval between milking and feed manipulation was non-linear (P = 0.3; Fig. 1). Delay between milking time and feed manipulation time was a predictor of post-milking standing time (Table 4). The longest post-milking standing times were observed when feed was manipulated in the hour before milking or in the immediate time period after milking. Parity was associated with both pre- and post-milking standing time; older cows spent more time standing both before and after milking than younger cows.

3.3. Factors associated with eSCC

A total of 591 pairs of milk samples (i.e., consecutive DHI samples) were collected throughout the study (Table 5). Eighty-seven pairs of milk samples were excluded due to high initial SCC (i.e., SCC on first sample within a pair was >100,000 cells/mL) resulting in 504 pairs of samples enrolled for the analysis of eSCC. From these paired samples, 48 cases of new eSCC were detected. Thus, the mean herd eSCC incidence rate was 0.91 eSCC/cow-year (SD: 0.49 eSCC/cow-year).

The incidence of eSCC across the range of average post-milking standing duration is presented in Fig. 2. Incidence of eSCC peaked in those cows that stood for approximately 80 min post-milking with the incidence of eSCC tending to decrease after this cut point. Non-linearity of the relationship between average post-milking standing time and risk of a new eSCC was clear (P = 0.8; Fig. 3). This allowed
Table 3
Final general linear model for factors associated with lying behavior of free-stall housed cows.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lying time (h/d)</th>
<th>Lying bout frequency (bouts/d)</th>
<th>Lying bout duration (min/bout)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β&lt;sup&gt;b&lt;/sup&gt;</td>
<td>SE</td>
<td>P value</td>
</tr>
<tr>
<td>Intercept</td>
<td>11.7</td>
<td>0.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DIM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.009</td>
<td>0.002</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>Ref&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-0.52</td>
</tr>
<tr>
<td>≥3</td>
<td>-</td>
<td>-</td>
<td>-0.82</td>
</tr>
<tr>
<td>Milk yield, kg/d</td>
<td>-0.046</td>
<td>0.013</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lameness&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>Ref</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yes</td>
<td>0.75</td>
<td>0.023</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are averaged across the recording periods for each of the 40 focal cows from each of the 5 herds (with the exception of 39 cows at one herd) that were observed for 3 observation periods (4 periods for 2 herds, 3 periods for 2 herds, and 1 period for 1 herd) with 35 d intervals between periods.

<sup>b</sup> β = estimated regression coefficient.

<sup>c</sup> DIM = days in milk at the beginning of each observation period.

<sup>d</sup> Ref = Reference category.

<sup>e</sup> Lameness: no = gait score <3; yes = gait score ≥3. Gait score was assessed on a 5-point scale (1 = normal to 5 = severely lame) (Flower and Weary, 2006; Ito et al., 2010).

Table 4
Final general linear model for factors associated with pre- and post-milking standing time of free-stall housed cows.<sup>a</sup>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-milking standing time (min)</th>
<th>P value</th>
<th>Post-milking standing time (min)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β&lt;sup&gt;b&lt;/sup&gt;</td>
<td>SE</td>
<td></td>
<td>β</td>
</tr>
<tr>
<td>Intercept</td>
<td>101.6</td>
<td>6.4</td>
<td>&lt;0.001</td>
<td>96.1</td>
</tr>
<tr>
<td>Milking-feeding interval&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤60 min</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-11.1</td>
</tr>
<tr>
<td>&gt;60 to 0 min</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-0.5</td>
</tr>
<tr>
<td>&gt;0 min</td>
<td>-</td>
<td>-</td>
<td>Ref&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>DIM&lt;sup&gt;d&lt;/sup&gt;</td>
<td>-0.18</td>
<td>0.04</td>
<td>&lt;0.001</td>
<td>-</td>
</tr>
<tr>
<td>Parity</td>
<td>Ref</td>
<td>-</td>
<td></td>
<td>Ref</td>
</tr>
<tr>
<td>1</td>
<td>9.3</td>
<td>5.7</td>
<td>11.6</td>
<td>6.4</td>
</tr>
<tr>
<td>2</td>
<td>12.3</td>
<td>5.7</td>
<td>14.7</td>
<td>6.3</td>
</tr>
<tr>
<td>≥3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are averaged across the recording periods for each of the 40 focal cows from each of the 5 herds (with the exception of 39 cows at one herd) that were observed for 5 observation periods (4 periods for 2 herds, 3 periods for 2 herds, and 1 period for 1 herd) with 35 d intervals between periods.

<sup>b</sup> β = estimated regression coefficient.

<sup>c</sup> Minimum difference in time between when cows were milked and when fresh feed was provided.

<sup>d</sup> Ref = Reference category.

Table 5
Number of pairs of milk samples taken per farm and number of newly elevated somatic cell count (eSCC) across the study period<sup>a</sup>.

<table>
<thead>
<tr>
<th>Herd</th>
<th>Total pairs of samples</th>
<th>Total pairs at risk&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Number of new eSCC&lt;sup&gt;c&lt;/sup&gt;</th>
<th>eSCC incidence rate (eSCC/cow-year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>118</td>
<td>108</td>
<td>12</td>
<td>1.13</td>
</tr>
<tr>
<td>2</td>
<td>117</td>
<td>91</td>
<td>15</td>
<td>1.58</td>
</tr>
<tr>
<td>3</td>
<td>160</td>
<td>133</td>
<td>7</td>
<td>0.51</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>39</td>
<td>2</td>
<td>0.37</td>
</tr>
<tr>
<td>5</td>
<td>156</td>
<td>133</td>
<td>12</td>
<td>0.96</td>
</tr>
<tr>
<td>Total</td>
<td>591</td>
<td>504</td>
<td>48</td>
<td>0.91</td>
</tr>
</tbody>
</table>

<sup>a</sup> Data are averaged across the recording periods for each of the 40 focal cows from each of the 5 herds (with the exception of 39 cows at one herd) that were observed for 5 observation periods (4 periods for 2 herds, 3 periods for 2 herds, and 1 period for 1 herd) with 35 d intervals between periods.

<sup>b</sup> Pairs were considered at risk when SCC <100,000 cells/mL at the beginning of each observation period.

<sup>c</sup> New eSCC was defined as SCC >200,000 cells/mL at the end of each period when SCC <100,000 cells/mL at the beginning of each period.

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For more meaningful categories to be chosen for average post-milking standing times based on variable distribution and on existing knowledge concerning teat canal closure following milking (McDonald, 1975; Schultz and Bright, 1983). Two categories of average post-milking standing time were defined: 0–90 min and >90 min based on the distribution of post-milking standing durations (where the median post-milking standing time was 92 min) and the observed relationship between post-milking standing time and incidence of eSCC (Figs. 2 and 3). Unconditional estimates of association between independent explanatory factors and odds of having an incident eSCC are described in Table 6. Post-milking standing time >90 min, encouraging post-milking standing time through
Table 6
Unconditional estimates of association between explanatory factors and odds of having an incident elevated somatic cell count.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Percentage or mean (±SD)</th>
<th>Odds ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>26.0</td>
<td>Ref</td>
<td>0.007</td>
</tr>
<tr>
<td>≥2</td>
<td>40.7</td>
<td>5.31 (1.86, 15.19)</td>
<td></td>
</tr>
<tr>
<td>LnSCC (days)</td>
<td>33.3</td>
<td>2.99 (1.02, 8.75)</td>
<td>0.09</td>
</tr>
<tr>
<td>DIM (days)</td>
<td>113.5 (50.9)</td>
<td>1.31 (0.95, 1.81)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
| Encourage post-milking standing
during milking | 3.44 (0.63) | 2.13 (1.51, 3.02) | 0.035   |
| Post-milking standing time |                          |                     |         |
| 0–90 min                  | 49.2                     | Ref                 |         |
| >90 min                   | 50.8                     | 0.47 (0.23, 0.95)   |         |
| Lamenessa                 |                          |                     |         |
| No                        | 64.1                     | Ref                 | 0.09    |
| Yes                       | 35.9                     | 0.40 (0.18, 0.92)   |         |
| Udder hygiene             |                          |                     |         |
| No                        | 88.9                     | Ref                 |         |
| Yes                       | 11.1                     | 1.27 (0.97, 1.67)   |         |
| Teat dip method           |                          |                     |         |
| Dip cup                   | 53.5                     | Ref                 |         |
| Foam                      | 46.5                     | 1.81 (0.72, 4.60)   |         |
| Stall width (cm)          | 121.7 (6.0)              | 0.70 (0.46, 1.07)   | 0.10    |

* Data are averaged across the recording periods for each of the 40 focal cows from each of the 5 herds (with the exception of 39 cows at one herd) that were observed for 5 d observation periods (4 periods for 2 herds, 3 periods for 2 herds, and 1 period for 1 herd) with 35 d intervals between periods.

* Odds ratio and 95% confidence interval for 1 SD increase for continuous variables presented.

* Reference category.

* LnSCC = natural log of individual cow SCC (×1000 cells/mL) at the beginning of the study period.

* Odd ratio and 95% confidence interval for one standard deviation increase in initial LnSCC.

4. Discussion

The existence of an association between post-milking standing time and udder health, in terms of SCC and infection rates, has long been hypothesized. A study of tie-stall housed cows by DeVries et al. (2010) provided the first direct empirical evidence of an association between post-milking standing time and incidence of IML. Evidence for this relationship in free-stall housed cows milked with an AMS was also recently reported (DeVries et al., 2011a). To our knowledge, this is the first study to report the association between post-milking standing duration and udder...
health, of free-stall housed cows that are parlor milked 3×/d. Mean post-milking standing time across all 5 herds was 103 min (range: 1–798 min), which is much longer than previously reported for free-stall housing systems milking 2×/d in a parlor, range 33–62 min, (Tyler et al., 1997; DeVries and von Keyserlingk, 2005; DeVries et al., 2005) or milking 2.3×/d in an AMS (78 min: DeVries et al., 2011a). In addition, the present duration of post-milking standing time was also much longer than the 79 min reported in the tie-stall study by DeVries et al. (2010). The average duration of pre-milking standing of 87 min was comparable to results from free-stall housed cows milked by an AMS (94 min: DeVries et al., 2011a). Given the duration of average pre-milking standing time, the duration of the post-milking standing times observed in the present study were unexpected, as one could hypothesize that cows would lie down sooner post-milking given their increased pre-milking standing time demands when milked 3×/d. While we did not collect data on time spent in the holding pen prior to being milked, one possible explanation for the long pre-milking standing times observed in these cows could be that parlor milked, free-stall housed cows often have a forced standing period in the holding pen of 30 min to >1 h prior to being milked (Cook and Nordlund, 2009). A more recent study of North American free-stall farms which were milking either 2 or 3×/d found that the average amount of time cows were spending away from their pens for milking was 249 min, indicating that these cows are likely spending long periods of time in the holding pen waiting to be milked (von Keyserlingk et al., 2012). Despite the pre-milking standing time of the study cows, indicating that they are likely spending long periods of time in the holding pen waiting to be milked, their post-milking standing times did not appear to be reduced. Also, across the study farms, higher producing animals (milk production >36.8 kg/d) stood on average for 12 min longer post-milking than the lower producing focal cows. Therefore, a possible explanation for the duration of post-milking standing time would be that these cows may have greater metabolic demands as a consequence of the higher milk production normally observed with increased frequency of milking and thus increased feeding motivation (O’Driscoll et al., 2010).

It is well established that the presence of fresh feed promotes longer post-milking standing time in cows (Tyler et al., 1997; DeVries and von Keyserlingk, 2005; DeVries et al., 2010). Given the variation in both the frequency of feed delivery (1 to 2×/d) of fresh feed and feed push-up (0 to 5×/d) across farms it was not surprising that feed manipulation (both feed delivery and push-up) was associated with post-milking standing time. On average feed manipulation occurred 62 min before milking with post-milking standing times reduced when feed manipulation occurred >60 min before milking. The longest post-milking standing times were observed when cows were fed in the hour before milking or in the short period of time after the return from milking (DeVries and von Keyserlingk, 2005; DeVries et al., 2010, 2011a).

The average daily lying time of 11.2 h/d was comparable to the average lying time reported for 16 free-stall herds milked either 2×/d or 3×/d (1.9 h/d; Gomez and Cook, 2010) and was comparable to the lying time reported in a study on high-producing Holsteins from North American free-stall herds (von Keyserlingk et al., 2012). However, the average lying times for the 5 herds observed in the present study were slightly higher than that reported by Calderon and Cook (2011: 3×/d milked herd; 9.8–10.8 h/d) and lower than the 13 h/d reported by Krawczel et al. (2012). The average number of lying bouts (8.6 bouts/d) and average bout duration (84.6 min/bout) were also similar to other studies observing 2×/d milked herds (Krohn and Munksgaard, 1993; Ito et al., 2009). However, the reduced lying bouts observed in our study compared to previous work (12.9 bouts/d; Gomez and Cook, 2010; 12.5 bouts/d; Krawczel et al., 2012) is difficult to explain. Previous work has speculated that cows increase their bout frequency in relation to increased milking frequency but we failed to show this effect. Rather it appears that cows with long post-milking standing times compensate by increasing the bout duration of their lying and standing events, thus decreasing the total number of daily lying and standing bouts. The
reduction in lying bout frequency may also be the result of cows prioritizing their feeding times to coincide with milking times, this would also explain the long post-milking standing times of the cows in this study. Daily lying times of cows from farms which provided >0.66 m/cow at the bunk averaged 11.3h/d compared to 10.9h/d on farms which provided <0.66 m/cow. This would indicate that despite an increased number of milkings per day, 3×/d milked cows are driven to maintain long daily lying durations and that adequate space at the feed bunk may enable them to economize feeding time, thus leading to fewer but longer lying bouts per day. Our finding that DIM was associated with all facets of lying behavior confirms the observational results previously reported by Bewley et al. (2010), who found that DIM was a predictor of lying duration with lying duration increasing in later lactation. Longer lying durations in later lactation may be a result of lessened metabolic demands as lactation progresses; therefore, cows may be spending less time feeding and spending more time engaged in resting behaviors (Bewley et al., 2010). Increased lying times observed in later lactation cows may also be a result of decreased udder fill. Österman and Redbo (2001) observed that morning pre-milking standing time was reduced in cows milked 2×/d as opposed to cows milked 3×/d (15 vs 8 h time between milking). These latter researchers hypothesized that this decrease in lying behavior before the morning milking in cows milked 2×/d was a result of discomfort caused by increased udder fill and pressure. When comparing production, DIM, and daily lying times across this study, cows producing between 30 and 40 kg/d tended to be higher in DIM compared to cows producing between 40 and 50 kg/d and those producing in excess of 50 kg/d (127 d vs 98 d and 80 d, respectively) and as production increased with decreasing DIM, average daily lying time also decreased from 11.2 h/d to 10.1 h/d. Thus, it is plausible that greater milk production in early lactation and consequently greater udder fill, may also result in reduced lying times compared to later lactation cows.

Parity was negatively associated with lying bout frequency, with cows of greater parity having fewer lying bout/d. This may be driven in part by the higher milk yield normally observed in multiparous cows (DeVries et al., 2011b); first lactation cows in the current study were producing on average 31 kg/d compared to 39 kg/d for the multiparous cows. Again the higher metabolic demands associated with elevated milk production in the older cows may have resulted in increased feeding times post milking (and thus longer post milking standing times).

In the current study, on average, 5.7% and 12.9% of primiparous and multiparous cows were considered to be clinically lame (gait score ≥3), respectively. It is well recognized that higher producing cows are at greater risk for lameness (Archer et al., 2010) and that lame cows have fewer lying bouts/d (Gomez and Cook, 2010). In concordance with previous work, lameness was associated with lying behavior in our study; lame cows had greater lying times (11 h/d for non-lame cows vs 12.4 h/d for lame cows) and lying bout duration (83 min/bout for non-lame cows vs 97 min/bout for lame cows). Calderon and Cook (2011) also observed an increase in lying times in lame cows that were parlor milked 3×/d in a free-stall barn. Lame animals likely have an increased daily lying duration and longer bout durations in an effort to mitigate pain they may be experiencing when standing (Chapinal et al., 2009).

The study farms selected were representative of commercial Ontario dairy herds in terms of milk quality. The average bulk milk SCC of 247 ± 106 (× 1000) cells/mL was similar to that reported for Ontario (205,000 cells/mL; Olde Riekerink et al., 2008). The 0.91 eSCC/cow-year incidence rate observed in this study was lower than recent studies reporting IMI incidence rate for the same geographic region (1.45 new IMI/quarter-year: DeVries et al., 2010; 1.95 new IMI/quarter-year: DeVries et al., 2011a). This observed difference may be the result of differing sensitivities between the two methods; SCC vs bacteriological sampling. SCC is an effective screening tool to identify potential infections, but fails to explicitly confirm IMI status. Moreover, older cows naturally have higher SCC levels and there is a dilution effect when assessing cow-level SCC as the inflammatory process is at the quarter-level (Bradley and Green, 2005). While SCC may be a less sensitive method of identifying IMI, it is a more practical and cost-effective method (Pyörälä and Pyörälä, 1997), and it is routinely measured on commercial farms. In subsequent studies, bacteriological culture of aseptically collected milk samples should be used to identify IMI and causal pathogens and validate eSCC measures.

Parity, baseline SCC, and post-milking standing time were the only variables retained in the final model as factors associated with eSCC. Higher parity cows are more likely to have pendulous udders and thus poorer udder hygiene (Renée et al., 2005). Milk production and mastitis have also been linked with parity, with mastitic cows having higher pre-mastitis milk yields compared to non-mastitic cows (Hagnestam et al., 2007). With increased milk yield comes increased metabolic demands and, thus, increased time spent standing to feed. As multiparous cows tend to be higher producers, they likely spend more time standing at the feed bunk and are thus at greater risk of becoming dirty (DeVries et al., 2011a; Nielsen et al., 2011). In fact, those animals in third lactation or higher had a numerically poorer udder cleanliness score (2.5 vs 2.4) compared to cows in their first or second lactation. Further, primiparous cows stood on average 1 h less than multiparous cows. Thus, higher parity cows may be at increased odds of eSCC because of reduced cleanliness, as a result of increased standing time and lower udder carriage, which increases the risk of exposure of the teat ends to manure from the alley floor.

Parity has also been linked with baseline SCC, with multiparous cows naturally having a higher SCC (Olde Riekerink et al., 2007). Even though there was no interaction between parity and baseline SCC on the risk of eSCC, the higher baseline SCC was, in addition to parity, associated with higher risk of eSCC. For the purposes of this study, a SCC level of >200,000 cells/mL was considered an eSCC, and used as a proxy for potential infection, while an SCC <100,000 cells/mL was considered normal (Dohoo and Leslie, 1991; Bradley and Green, 2005). While cows needed to have a SCC <100,000 cells/mL to be considered at risk for acquiring a new eSCC, those cows which had an SCC closer to 100,000 cells/mL were at increased odds of
acquiring a new eSCC. A potential limitation of our study is the presence of false negatives; cows that were categorized as not infected according to our eSCC definition but in fact did have an IMI. Bradley and Green (2005) noted that while major pathogens cause SCC to increase above 200,000 cells/mL, minor pathogens have a lesser impact on SCC. In fact, SCC may be as low as 50,000 cells/mL even though a cow is infected. In future studies, bacteriological culture is recommended to help ensure that those animals selected as uninfected based on SCC were actually true culture-negative.

Cows with post-milking standing times averaging >90 min were at decreased odds for acquiring a new eSCC. Previous research (McDonald, 1975; Schultze and Bright, 1983) would suggest that there are 2 periods of time whereby teat canal diameter may be expanded and, thus, more susceptible to bacterial penetration following milking: immediately after removal of the milking machine and 2 to 4 h after milking. Given that in the current study few cows chose to lie down quickly after milking (only 6% of cows studied lay down within 40 min after milking), it is not that surprising that we did not detect any change in odds of incidence of eSCC in those cows. The decreased odds of eSCC with post-milking standing times >90 min in the current study contrasts to that found by DeVries et al. (2010, 2011a), who noted increased odds of IMI when cows spent >1.5 and 2.5 h, respectively, standing after milking. These latter authors speculated that the threshold for the second period of susceptibility might be at the lower end of the 2–4 h period after milking. In the current study, just over 23% of cows spent between 90 and 120 min standing after milking, 25% of cows spent between 2 and 4 h standing after milking, while only 3% stood for over 4 h after milking. These findings would suggest that the start of the second period of susceptibility might under certain conditions be closer to the 4 h mark after milking. Further research is required to fully understand these factors that may affect teat canal diameter at various time points post-milking, and the resultant influence this has on the risk of acquiring infections during those time periods.

5. Conclusions

Free-stall housed that are milked 3×/d that were in later lactation, produced less milk, or were lame had the longest daily lying times. Lying bout frequency was greater in older cows or cows in earlier lactation, while lying bout duration increased with DIM and in those cows identified as being lame. The results of the current study suggest that free-stall housed cows milked 3×/d that remain standing for greater than 90 min after milking are at decreased odds for acquiring a new eSCC compared to those cows that lay down within 90 min after milking. Provision of feed in the hour before milking, or in the time period immediately after milking, led to the longest duration of post-milking standing. Management practices that promote post-milking standing duration, such as providing new feed or pushing-up feed around milking time may help to improve udder health.

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References


