Relationship between feeding strategy, lying behavior patterns, and incidence of intramammary infection in dairy cows

T. J. DeVries,^{*1} S. Dufour,[†] and D. T. Scholl[†]

^{*}Department of Animal and Poultry Science, University of Guelph, Kemptville Campus, 830 Prescott Street, Kemptville, Ontario, K0G 1J0, Canada

[†]Faculty of Veterinary Medicine, University of Montreal, CP 5000, Saint-Hyacinthe, Quebec, J2S 7C6, Canada

ABSTRACT

The objectives of this study were to investigate whether feeding strategy influences post-milking standing time in dairy cows and to determine whether this time relates to incidence of intramammary infection (IMI). Fifteen lactating dairy cows (5 most recently fresh, 10 randomly chosen) from each of 6 tie stall dairy farms were enrolled for a total of 90 cows. Quarter samples of milk were taken from each cow once every 3 wk for a total of 3 samplings, and routine bacteriological culture and identification procedures were conducted. National Mastitis Council guidelines were used to define IMI, and occurrence of a new IMI was defined as a positive culture sample following a negative culture sample. Data on lying behavior patterns were collected using data loggers for every cow for 7 d before each milk sampling. For these 7 d, individual milking and feeding times of the cows were also recorded. Our results demonstrated that the provision of feed around milking time (between 30 min before and 60 min after) resulted in the longest post-milking standing times. The shortest post-milking standing times were seen in those cows that were fed >30 min before milking. Feeding cows >60 min after milking resulted in only slightly shorter post-milking standing times than those fed between 30 min before and 60 min after milking. Cows lying down for the first time 40 to 60 min after milking had 1.4 times lower odds of acquiring a new environmental IMI than cows lying down within 40 min after milking. As post-milking standing time increased past 60 min, the odds of acquiring a new environmental IMI increased as well. The cows lying down for the first time 60 to 90, 90 to 120, and >120 min after milking showed 3.2, 5.8, and 7.4 times higher odds, respectively, of acquiring a new environmental IMI compared with cows lying down for the first time within 40 min of milking. Our results suggest that despite being able to manage post-milking standing times by providing fresh feed at different times

around milking, the use of such a feeding strategy in tie stall systems is an unrealistic IMI prevention strategy.

Key words: dairy cow, intramammary infection, lying behavior, feeding strategy

INTRODUCTION

Researchers have shown that the presence of feed, particularly fresh feed, around milking will promote longer post-milking standing times in dairy cows (Schultz, 1985; Tyler et al., 1997; Johansson et al., 1999; DeVries and von Keyserlingk, 2005). The practical significance of this increase in latency to lie down following milking is not fully understood. The common belief is that the longer the animal stands after milking, the lower the risk for bacterial penetration of the teat orifice when the cow eventually lies down. One of the reasons behind this is the fact that the relative penetrability and diameter of the orifice are increased for a time after milking (McDonald, 1975; Schultze and Bright, 1983); therefore, preventing the teat end from contacting the lying surface during that time period could potentially reduce the risk of acquiring new IMI.

There is some evidence from epidemiological studies that promoting longer standing time in cows following milking is associated with a lower herd SCC. In a study of French dairy herds, Barnouin et al. (2004) found that those herds that locked up cows at the feed-line after milking, and thus prevented them from lying after milking, were more likely to be classified as having very low SCC. In a survey of low-SCC herds in Britain, Peeler et al. (2000) found that the incidence of clinical mastitis was lower on farms that offered fresh feed after both the morning and evening milking. Further, a study on Dutch dairy farms found that those farmers whose management styles were identified as clean and accurate prevented cows from lying down after milking (Barkema et al., 1999).

To date, no specific studies have investigated how the effect of post-milking standing time influences the risk of infection of the udder by pathogens. Further, the relationship between feed provision and post-milking standing time across a continuum of feeding times rela-

Received September 2, 2009.

Accepted January 4, 2010.

¹Corresponding author: tdevries@kemptvillec.uoguelph.ca

Table 1. Descriptive summary of the cows sampled within each study herd at the beginning of the study period¹

Herd	Cows sampled/herd	Mean parity	Mean DIM	Mean 305-d milk production (kg)	Mean SCS ²
1	15	2.5	123	10,870	1.3
2	15	2.7	102	10,554	0.7
3	15	2.3	138	12,027	1.2
4	14 ³	2.9	121	9,912	0.5
5	15	1.7	96	9,278	0.2
6	15	2.4	123	10,458	0.4

¹Means are based on data from 5 most recently fresh and 10 randomly selected cows from each study herd.

²As described by Shook (1993).

³One of the randomly selected cows was removed from this herd after the beginning of the study.

tive to milking is unknown. Thus, the objectives of this study were to evaluate how the timing of feed provision affects post-milking standing time and to determine how this standing time following milking relates to the incidence of IMI in lactating dairy cows. The hypothesis was that provision of feed closest to the time of milking promotes the longest post-milking standing times and that cows that lie down for the first time soonest after milking would be at most risk of acquiring an IMI caused by an environmental pathogen.

MATERIALS AND METHODS

Sample Size Estimation

Because no method for sample size estimation for multilevel logistic regression has been published, the method proposed by Broll et al. (2002) for conventional logistic regression models was used to provide an approximate needed sample size. Using PASS software (PASS, Kaysville, UT), it was determined that a sample size of 582 pairs of milk samples could achieve 80% power at a 0.05 significance level to detect a difference in risk of IMI corresponding to an odds ratio greater than 1.5 or smaller than 0.67 for every standard deviation increase of average post-milking standing time. Overall, we aimed for 720 pairs of milk samples to account for expected lost/contaminated samples, culled cows, and possible differences in power when using a multilevel logistic regression.

Farm Selection

Six dairy farms participating in the National Cohort of Dairy Farms of the Canadian Bovine Mastitis Research Network (Reyher et al., 2009) from Ontario, Canada, were chosen for use in this study. Herds were selected on the criteria that they were all tie stall herds that milked twice per day, were predominantly (>80%) Holstein, and used similar straw bedding systems. Overall, these farms had a mean herd size of 75

(range: 49–110) cows and a mean adjusted 305-d milk yield of 10,756 kg (range: 10,370–11,346 kg). The mean annual bulk milk SCC was 276,000 cells/mL (range: 189,000–402,000 cells/mL). During the year of study, the mean incidence of clinical mastitis cases on these farms was 43 cases per 100 cow-years (range: 15–62 cases/100 cow-years). All cows on each farm were managed according to the guidelines set by the Canadian Council on Animal Care (1993).

Milk Sampling and Analysis

A subsample of 15 lactating cows (5 most recently fresh and 10 randomly chosen) from each farm (Table 1) were milk sampled and resampled twice at 3-wk intervals for a total of 3 samplings from June 2008 to August 2008. This sampling involved taking milk samples by sterile technique from each quarter of each animal (Hogan et al., 1999). Before sampling, the first streams of milk were discarded and teat ends were disinfected with cotton swabs soaked in 70% alcohol and allowed to dry. Then, approximately 30 mL of milk was drawn into a sterile container. These samples were frozen at –20°C and later thawed for milk bacteriological culture and for SCC determination.

Bacterial culture was performed to identify pathogens, including *Escherichia coli*, *Klebsiella* spp., *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, and other *Streptococcus* spp., as well as CNS, *Corynebacterium* spp., and *Staphylococcus aureus*. This was conducted by following standardized protocols based on National Mastitis Council guidelines for bacteriological culture and species identification (Hogan et al., 1999). Ten microliters of milk were streaked onto blood and MacConkey biplates and aerobically incubated at 35°C for 24 h. Colonies were enumerated (up to 10 colonies or 10+ colonies) and speciated after 24 h, and were then reincubated for a further 24 h. After 48 h, bronopol was added to the samples and they were refrozen in preparation for shipping to the SCC laboratory (Maritime Quality Milk, Charlottetown,

Prince Edward Island). Somatic cell count analysis was conducted using the Fossomatic method (Fossomatic 4000 series, Foss Electric, Hillerød, Denmark).

Samples from which 3 or more pathogens were isolated, except those that included *Staphylococcus aureus* or *Streptococcus agalactiae*, were classified as contaminated and were therefore excluded from subsequent analyses. The National Mastitis Council guidelines were used for definition of an IMI at the quarter level (Harmon et al., 1990). Occurrence of a new IMI in a quarter over a 3-wk observation period was defined as an infection-free sample at the beginning of the 3-wk period followed by a sample positive at the end of the period. Nonoccurrence of a new IMI was defined as 2 consecutive infection-free samples. Therefore, quarters had to be free of IMI at the first or second sampling to be considered at risk of a new IMI for the first or second period of observation. For the same reason, a quarter experiencing a new IMI during the first period of observation could not be considered at risk of becoming infected during the second period.

Standing and Lying Behavior

Standing and lying behaviors were collected using data loggers (HOBO Pendant G Data Logger, Onset Computer Corporation, Pocasset, MA). This device measures leg orientation at 1-min intervals and allowed all the standing and lying behavior data to be collected electronically. These devices were attached to one of the hind legs of the cows using veterinary bandaging tape (Vetrap Bandaging Tape, 3M, London, Ontario, Canada) 7 d before each of the milk sampling events and were removed following each milk sampling. Data collected were used to calculate standing and lying times (min/d), bout frequency (n/d), and bout length (min/bout).

Animal, Herd, Housing, and Management Data

During the week while lying behavior of the cows was being monitored, the individual daily times of milking for each animal and feed delivery were recorded by the producers. Prior to taking milk samples, the cleanliness of each of cow was scored by a research technician using a hygiene scoring system (www.vetmed.wisc.edu/dms/fapm/fapmtools/4hygiene/hygiene.pdf; Cook and Reinemann, 2007). Using this system, cows were scored on a 4-point cleanliness scale, evaluating lower legs, udder, and upper legs-flanks (1 = very clean to 4 = very dirty). Teat end roughness and callosity were also scored by the research technician at each sampling using the scale described by Neijenhuis et al. (2000). All other specific cow and lactation data were obtained

from the local DHI organization. Housing and management data, including stall dimensions, bedding (type, quantity, frequency of cleaning), and management style, were collected through on-farm observations and a validated udder health management questionnaire (Dufour et al., 2010).

Statistical Analysis

The time delay between milking time (a.m. and p.m.) and the closest-in-time delivery of feed (TMR, hay, or concentrates) were calculated. Post-milking standing time was calculated as the difference in time between the end of milking (a.m. and p.m.) and the first recorded instance when the cow laid down following milking. To determine the effect feed distribution had on post-milking standing time, a linear multilevel repeated measures mixed model was fitted to the data using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). In this model, the natural logarithm of post-milking standing time was treated as the outcome variable and delay between milking time and delivery of feed and measurement time were treated as fixed predictor variables. Milking was considered as a repeated measure. To account for correlation between cows within farm, a random farm effect was included in the model. To account for dependence between measurements made on the same cow and for the varying correlation between 2 measurements with increasing length of the time interval between the measurements, a random effect of cow within farm with a specific covariance structure was used. For this random effect, 4 covariance structures were compared: compound symmetry, first-order autoregressive, first-order autoregressive moving average, and Toeplitz. The Toeplitz structure was chosen on the basis of best fit using a likelihood ratio statistic, as described by Dohoo et al. (2003). The general model was as follows:

$$\text{PMST}_{ijk} = \beta_0 + \beta_1 \text{FEED}_{ijk} + \beta_2 \text{MILKING}_{ijk} + v_{0k} + u_{0jk} + e_{0ijk}, \quad [1]$$

where PMST_{ijk} is the predicted natural logarithm of post-milking standing time for the i th milking (milking 1, 2, and so on) of the j th cow from the k th herd; β_0 is the intercept; β_1 and β_2 are the regression coefficients for the delay between milking time and delivery of feed and measurement time (milking), respectively; and v_{0k} , u_{0jk} , and e_{0ijk} are the herd random effect, cow random effect, and measurement (one milking) error term, respectively (all assumed to follow an approximately normal distribution).

Parity (first-calf heifer or mature cow), DIM, and cow's milk production were introduced in model [1] one at a time to assess their significance and the possible presence of confounding bias on the estimated feeding strategy–post-milking standing time relationship. For the latter, the change in the estimate of the mean effect of the feeding strategy–post-milking standing time association was evaluated and variables causing a change of 10% or more were retained as important confounding variables. For all continuous predictors, linearity of the relationship was assessed and variables were categorized in a meaningful way whenever the assumption of linearity could not be met. Effect modification by variables of the feeding strategy–post-milking standing time association was tested by the significance in the model of an interaction term of the predictor variable and the other variable.

To determine the association between post-milking standing time and incidence of IMI, the cow's average post-milking standing time during the last 7 d of each sampling period was used. The hypothesized biological pathway through which post-milking standing time can affect udder health is through prevention of IMI by preventing entry through the nonclosed teat canal by bacteria present in the environment of the cow. Therefore, new IMI caused by bacteria species that are considered as being rather strictly contagious pathogens (*Staphylococcus aureus* and *Streptococcus agalactiae*) were not considered in these analyses because these IMI should not be affected by post-milking standing time. Multilevel models were fitted to the data using MLWin 2.10 (Rasbash, London, UK). A 3-level [period (1 pair of sequential samples), quarter, and cow] random intercept logistic model containing a constant and herd as fixed effects and with the dependant variable being occurrence or nonoccurrence of a new IMI other than *Staphylococcus aureus* or *Streptococcus agalactiae* was developed. The general model was as follows:

$$\begin{aligned}
 Y_{ijk} &\sim \text{Bin}(1, \pi_{ijk}) \\
 \text{Logit}(\pi_{ijk}) &= \beta_{0jk} + X_{ijk} \beta_{ijk} \\
 \beta_{0jk} &= \beta_1 + v_{0jk} + u_{0jk} \\
 v_{0jk} &\sim N(0, \Omega_v) \\
 u_{0jk} &\sim N(0, \Omega_u) \\
 \text{Var}(Y_{ijk} \mid \pi_{ijk}) &= \pi_{ijk}(1 - \pi_{ijk}), \quad [2]
 \end{aligned}$$

where Y_{ijk} is the odds of acquiring a new IMI other than *Staphylococcus aureus* or *Streptococcus agalactiae* for the i th pair of samples of the j th quarter from the

k th cow, it is a function of a series of fixed predictors (X) through the logit function, and it approximately follows a binomial distribution; X_{ijk} represents the fixed predictors (average post-milking standing time, herd effect, and covariables); β_1 is the intercept; v_{0jk} and u_{0jk} are the cow and quarter error terms, respectively (random part of the model), and are assumed to follow an approximately normal distribution with mean zero and variance Ω_v and Ω_u ; and π_{ijk} represents the probability that $Y_{ijk} = 1$.

Independent variables were screened using the described model from the bivariable analyses to identify potential covariates for multivariable modeling. The variables screened in the bivariable analyses included these herd-level variables: herd size, stall base (mattress or rubber mat), stall length and width, distance from neckrail to curb, neckrail height, bedding quantity (≤ 2 cm or > 2 cm), stall manure removal and dirty bedding removal frequency (times/d), part of the stalls cleaned (rear third, rear half, or complete stall), bedding addition frequency (times/d), frequency of complete removal and replacement of stall bedding (times/mo), and meticulousness of the herd manager and management style of the farm (rated by the research team on a 1-to-10 scale; for details, see Dufour et al., 2008). Cow-level variables were 305-d milk production and parity. Quarter variables were teat end roughness and callosity. Period variables were average post-milking standing time, average lying time per day, average number of lying bouts per day, individual quarter SCC natural logarithm at the beginning of the period, DIM at the beginning of the period, and cleanliness score of the lower legs, udder, and upper legs-flank at the beginning of the period. For variables measured on a continuous scale, linearity of the relationship between the variable and the log odds of the occurrence of a new IMI was assessed by categorizing the continuous variable and visual inspection of plots of the odds ratio against mean values of the categories. Meaningful categories were created whenever the assumption of linearity could not be met. Variables with $P \leq 0.20$ (Wald's test) were retained for further analyses.

Multivariable analyses were then performed. Because none of the quarter-level predictors were retained by the bivariable analysis and because our new IMI occurrence definition did not allow for within-quarter correlation, the quarter-level variance was null and, therefore, the multivariable analyses were realized using equation [2] without the quarter random effect term (u_{0jk}). Average post-milking standing time during the period was first introduced in this 2-level [period (1 pair of sequential samples) and cow] logistic model as our primary variable, with herd being treated as a fixed effect. The model building protocol was as documented above.

Figure 1. Moving average (with width of 60 min and increments of 30 min) of median (full line) and mean (dashed line) post-milking standing time across time delay between milking and feed delivery.

For all models, normality and homogeneity of the residuals were evaluated by examining the normal probability plots of residuals and plots of residuals against predicted values. All MIWin 2.10 multilevel analyses were performed using a restricted generalized iterative least squares algorithm and a second-order penalized quasi likelihood estimation. To provide revised estimates for the logistic model, a Bayesian Markov chain Monte Carlo estimation with diffuse sampling, a burn-in period of 500 iterations, and a run of 30,000 iterations was used.

RESULTS

Feeding Strategy–Post-Milking Standing Time Association

One cow was removed from a herd after the first milk sampling was performed, thus reducing our sample size to 89 cows; 1 cow was culled and another dried off after the first pair of milk samples was collected. A total of 3,543 post-milking standing time observations were obtained from these 89 cows. Post-milking standing time distribution was right skewed and the median time to first recorded instance of the cow laying down following milking was 64 min (mean: 78.6 min; range: 3–571 min). Delay between milking and feed delivery followed an approximately normal distribution, with the feed being delivered on average 30 min before milking (range: 225 min before milking to 180 min after milking).

Relationship between cows' post-milking standing time and delay between milking and feed delivery is illustrated in Figure 1. This association was not linear and delay between milking and feed delivery was, therefore, categorized based on the observed distribution into feed delivery >120 min before milking, 120 to

Figure 2. Box and whisker plot of post-milking standing time across time delay between milking and feed delivery categories. Each box contains 50% of the data, the median (line), the mean (x), and the upper (75%) and lower (25%) quartiles. The whiskers indicate maximal and minimal values.

30 min before milking, 30 min before to 60 min after milking, and >60 min after milking. The distribution of post-milking standing time across categories of delay between milking and feed delivery is illustrated in Figure 2. Delay between milking and feed delivery was a significant predictor of post-milking standing time (Table 2). The longest post-milking standing time was obtained from cows fed 30 min before to 60 min after milking. There was no significant association ($P > 0.05$) of parity, DIM, or cow's milk production with post-milking standing time, there was no evidence of confounding bias by these variables, and no significant interactions between these variables and the delay between milking and feed delivery were found.

Figure 3. Moving average (with width of 60 min and increments of 15 min) of IMI incidence rate across average post-milking standing time. Intramammary infection incidence rate (full line) with 90% (dashed lines) and 95% (dotted lines) confidence limits.

Table 2. Association between milking–feeding delay and post-milking standing time (min)

Timing of feed delivery	Mean	95% CI	P-value
>120 min before milking	62.4 ^a	41.4, 83.5	<0.001
120 to 30 min before milking	70.7 ^a	50.7, 90.6	<0.001
30 min before to 60 min after milking	86.5 ^b	66.6, 106.5	<0.001
>60 min after milking	81.5 ^b	59.7, 103.3	<0.001

^{a,b}Least squares means within a column with different superscripts differ ($P \leq 0.05$, adjusted for multiple comparisons using the Tukey-Kramer procedure).

Average Post-Milking Standing Time–IMI Incidence Association

Two milk samples were lost, resulting in a total of 702 pairs of milk samples taken from a total of 355 quarters (Table 3). Of these samples 116 pairs were not at risk of becoming infected (already infected on first or second sample or one of the samples was contaminated), leaving 586 pairs of samples at risk of becoming infected and 321 quarters studied. From these paired samples we detected 49 new IMI, including 29 new CNS IMI, 10 new *Corynebacterium* spp. IMI, 5 new *Staphylococcus aureus* IMI, 3 new *Streptococcus* spp. IMI (other than *Streptococcus agalactiae*, *uberis*, *dysgalactiae*, and *canis*), 1 new *Streptococcus uberis* IMI, and 1 new *Klebsiella* spp. IMI. The mean herd IMI incidence rate was 1.45 new IMI/quarter-year (SD: 0.30 IMI/quarter-year).

The relationship between IMI incidence rate (excluding *Staphylococcus aureus* or *Streptococcus agalactiae*) and post-milking standing time is illustrated in Figure 3. A decreasing IMI incidence rate was observed up to 45 min of average post-milking standing time followed by a gradually increasing IMI incidence rate, reaching a plateau around value of 100 min. Nonlinearity of the relationship between average post-milking standing time

and IMI incidence was clearly apparent as shown in Figure 4. Meaningful categories were therefore chosen for average post-milking standing times based on variable distribution and on existing knowledge of teat canal closure following milking (McDonald, 1975; Schultze and Bright, 1983). Five categories were defined: average post-milking standing times of 0 to 40 min, 40 to 60 min, 60 to 90 min, 90 to 120 min, and >120 min.

The independent variables retained by the bivariable analyses as well as their unadjusted association with IMI incidence are presented in Table 4. Two independent variables, 305-d milk production and cleanliness score of the lower legs, were retained as confounders of the average post-milking standing time–IMI incidence association based on percentage of change of the odds ratio. None of the tested interactions and random coefficients were retained in the final model. Adjusted estimates obtained from the final model showed a significant increased risk of acquiring a new IMI as post-milking standing time increased past 60 min (Table 5). The adjusted estimates of the fixed effects obtained using the Bayesian Markov chain Monte Carlo estimation estimations were in close agreement with those obtained from the penalized quasi likelihood estimation (data not shown).

DISCUSSION

The amount of time spent standing following milking has been widely hypothesized, with very little scientific support to date, to affect the risk of new IMI in lactating dairy cows. In fact, a limited number of

Figure 4. Adjusted estimates of the odds of acquiring a new IMI across deciles of average post-milking standing time (solid line) with 90% (dashed lines) and 95% (dotted lines) confidence limits.

Table 3. Number of pairs of quarter milk samples taken per farm and number of new IMI

Herd	Total pairs of samples	Usable pairs of samples	Number of new IMI	IMI incidence (IMI/quarter-year)
1	120	90	8	1.54
2	120	110	11	1.74
3	118	101	7	1.20
4	112	102	9	1.53
5	120	88	9	1.78
6	112	95	5	0.91

Table 4. Unadjusted odds-ratio (OR) estimates of fixed-effect associations with IMI incidence for variables retained after bivariable analysis

Variable	Percentage or mean (SD) ¹	OR (95% CI)	<i>P</i> -value ²
Post-milking standing time (min)			0.01
0–40	21.6	Reference	
40–60	19.7	0.85 (0.12, 6.1)	
60–90	23.3	3.9 (0.90, 17.3)	
90–120	18.4	9.2 (1.8, 45.8)	
>120	17.0	8.6 (1.8, 41.5)	
305-d adjusted milk production (kg)	10,483 (1,834)	1.4 (1.0, 2.1) ³	0.05
Cleanliness score of the lower legs			<0.01
1 or 2	91.4	Reference	
3 or 4	8.6	3.9 (1.5, 10.2)	

¹Proportion of observations for categorical variables or mean and standard deviation for continuous variables.²Joint *P*-value is given for multiple-class categorical variables.³Odds ratio and 95% confidence interval for one standard deviation increase (1,834 kg) of 305-d milk production.

researchers have actually calculated the amount of time dairy cows spend standing following milking. To our knowledge, this is the first report of the post-milking standing time calculated for cows that were individually housed (in tie stalls). In the current study, the mean post-milking standing time was 78.6 min. This is much longer than that previously reported, on average, for free stall-housed cows [34.5 min (Tyler et al., 1997); 55 min (DeVries and von Keyserlingk, 2005); 62 min (DeVries et al., 2005); and 32.5 min (Fregonesi et al., 2007)]. It could be hypothesized that the decision to lie down following milking is influenced by different factors in these different housing systems. For free stall systems, the decision to lie down would be affected by cows' motivation to access feed when present versus

to lie down in a stall. For example, Fregonesi et al. (2007) found that post-milking standing time decreased from 39 to 26 min when stocking density at the lying stalls was increased from 100 to 150%, suggesting that increasing competition for the stalls resulted in more cows choosing to lie down rather than go and feed. It is not unreasonable to assume that this motivation would also be influenced by competition for feed bunk access or the health status of the cow (e.g., lameness). These factors may not play as large of a role in the decision to lie down following milking for tie stall cows, which have their own feeding and lying area. Given this, it is not surprising that the post-milking standing time would be longer, as observed, for tie stall-housed cows. Another important difference between these housing

Table 5. Multilevel logistic model of the adjusted association between average post-milking standing time and occurrence of IMI (second-order penalized quasi likelihood estimation)

Variable	Coefficient	SE	Odds ratio (95% CI)	<i>P</i> -value ¹
Intercept	−5.688	1.430	—	—
Cow-level variance	0.192	0.352	—	—
Herd				0.56
1	Reference	Reference	Reference	
2	−0.193	0.595	0.82 (0.26, 2.6)	
3	0.392	0.749	1.5 (0.34, 6.4)	
4	0.736	0.657	2.1 (0.58, 7.6)	
5	−0.121	0.651	0.89 (0.25, 1.2)	
6	−0.373	0.854	0.69 (0.13, 3.7)	
Post-milking standing time (min)				0.01
0–40	Reference	Reference	Reference	
40–60	−0.314	0.985	0.73 (0.11, 5.0)	
60–90	1.156	0.742	3.2 (0.74, 13.6)	
90–120	1.751	0.821	5.8 (1.2, 28.8)	
>120	1.983	0.787	7.4 (1.6, 34.0)	
305-d milk production (kg)	0.00017	0.00011	1.4 (0.92, 2.0) ²	0.14
Cleanliness score of the lower legs				0.03
1 or 2	Reference	Reference	Reference	
3 or 4	1.210	0.541	3.4 (1.2, 9.7)	

¹Joint *P*-value is given for multiple-class categorical variables.²Odds ratio and 95% confidence interval for one standard deviation increase (1,834 kg) of 305-d milk production.

systems and possibly an important factor determining post-milking standing time is the amount of time cows will have already spent standing before being milked. Typically, in a free stall system, cows will be forced to stand and leave their pen to be brought to a waiting area, where they will usually have to stand for a certain amount of time before finally being milked and released back to their pen. Conversely, in a tie stall system, cows will be prompted to stand up only minutes before milking for milking preparation.

The one motivating factor to keep cows standing following milking, which both housing systems have in common, would be the presence of feed in the feed bunk. Previous research with free stall-housed cows has shown that the presence of feed, particularly that of freshly delivered feed, will keep cows standing for longer periods of time following milking (Tyler et al., 1997; DeVries and von Keyserlingk, 2005). One could assume that for tie stall-housed cows the provision of feed around milking time would be the primary factor influencing their decision to lie down following milking. The herds used in this study were all milked twice daily and provided fresh feed at least twice daily. As result, feed was typically provided around the times of milking (on average 30 min prior), but this did vary from 3.75 h before to 3 h after milking across the herds and observation days. Based on our assumption of the relationship between cows' average post-milking standing time and provision of feed, it was hypothesized that post-milking standing time would decrease as the delay between milking and feed delivery increased, both before or after milking. In support of our hypothesis, we did find that the provision of feed around the time of milking (between 30 min before and 60 min after milking) resulted in the longest post-milking standing times. In addition, as seen in Figure 2, the distribution of post-milking standing time for cows fed around milking was clearly different than when cows were fed at different times. For this group, fewer cows decided to lie down for the first time shortly after milking and more cows reacted to the feeding strategy in a similar manner, lying down for the first time around the mean value of this group. Conversely, when cows were fed either >30 min before milking or >60 min after milking, a large proportion of cows chose to lie down shortly after milking whereas a few cows stayed up for very long period of time, resulting in the observed right-skewed distribution of post-milking standing time for these groups. Overall, there was a more similar and reliable behavioral response of post-milking standing time for those cows fed around milking time. Our observations are consistent with previous findings that provision of feed at the time of milking results in longer post-milking standing times in free stall-housed cows (DeVries and von Keyserlingk,

2005) and in lower percentages of cows lying down within the first hour after milking (Johansson et al., 1999). Given the effect provision of fresh feed has on stimulating feeding activity in dairy cows (DeVries and von Keyserlingk, 2005), it can be assumed that provision of feed in the time frame around milking will result in cows engaging in some level of feeding activity, and thus remain standing to do so.

Interestingly, the post-milking standing time did not completely change as expected as the delay between milking and feed delivery time increased. As predicted, the shortest post-milking standing times were seen in those cows that were fed >30 min before milking. Dissimilar to our prediction, feeding cows >60 min after milking resulted in only slightly shorter post-milking standing times than feeding cows between 30 min before and 60 min after milking. Consideration of the meal patterning of dairy cows helps explain why feed provision >30 min before milking would affect the post-milking standing time. The average meal duration for a dairy cow typically lasts from 40 to 45 min and the average time interval between meals would typically range from 90 to 170 min (DeVries et al., 2007, 2009). For those cows fed >30 min before milking, it could be hypothesized that they would engage in a meal following the provision of fresh feed. Given the length of the meal and the average time between meals, it is not surprising that these cows would be the least inclined to stay standing and feeding for as long of a time following milking. For those cows fed >60 min after milking, it is very possible that they anticipated the delivery of feed after milking and thus remained standing for almost as long as a time period as those fed closer to milking time. In a study by Johansson et al. (1999), the effect of feeding time relative to milking (1.5 h prior, at milking, and 1.5 h after milking) on when cows lay down following milking was assessed by looking at the percentage of cows lying within the first hour after milking. Contrary to our findings, these researchers found that the cows fed 1.5 h after milking had the highest percentage lying within the first hour after milking. One explanation for this difference would be that the cows fed 1.5 h after milking in the Johansson et al. (1999) study did not have any feed available to them from the time of milking to the time of feed provision. In our study, those cows fed >60 min after milking may or may not have had feed available to them during that first hour after milking. Adding to this, of the total number observations of feeding >60 min after milking [$n = 97$ (2.8%)], there were relatively few times when feed was provided later than 1.5 h after milking [$n = 19$ (0.5%)].

For the determination of the relationship between post-milking standing time and IMI incidence, we hoped that the farms selected for this study would be represen-

tative in terms of udder health of tie stall commercial dairy herds typically found in Canada and northeastern parts of the United States. The clinical mastitis incidence (43 cases/100 cow-years; 95% CI: 25–60 cases) on the study herds was not significantly higher than that previously reported for the province of Ontario (31.6 cases/100 cow-years), the geographic region where the study farms were located (Olde Riekerink et al., 2008). Similarly, the bulk milk SCC (average: 276,000 cells/mL; 95% CI: 197,000–355,000 cells/mL) of the study herd was not significantly higher than the recently reported average for Ontario (205,000 cells/mL). The relatively high incidence of clinical mastitis and high bulk tank SCC in these herds are tie stalls herds' characteristics that have been observed before (Olde Riekerink et al., 2008). The number of new IMI was similar across the study herds, with an average mean herd IMI incidence rate of 1.45 new IMI/quarter-year.

The relationship between average post-milking standing time and IMI incidence was clearly nonlinear and, thus, meaningful categories were chosen based on observations by McDonald (1975) and Shultz and Bright (1983). These researchers, respectively, noted a slow reduction of teat canal dilation or frequency of penetrability of the papillary duct up to 2 h following milking. This slow reduction was followed by an apparent increase of the teat canal diameter or relative penetrability of the papillary duct between 120 and 240 min following milking. In respect to their findings, we defined 4 categories of post-milking standing time within the first 120 min following milking and a fifth category was defined as post-milking standing time of >120 min following milking.

The final multilevel logistic model revealed a strong significant relationship between post-milking standing time and occurrence of a new IMI. As hypothesized, cows lying down for the first time 40 to 60 min following milking had 1.4 times lower odds of acquiring a new IMI compared with cows lying down within 40 min from milking (our reference group); however, these differences were not significant. Contrary to what was expected, as post-milking standing time increased beyond 60 min, the odds of acquiring a new IMI increased as well. The cows lying down for the first time 60 to 90, 90 to 120, and >120 min after milking showed 3.2, 5.8, and 7.4 times higher odds, respectively, of acquiring a new IMI compared with cows lying down for the first time within 40 min of milking (with results from the last 2 categories being significantly different from the reference group).

Although these results seem surprising, they are in complete agreement with current knowledge of teat canal closure. McDonald (1975) identified 2 different periods of time whereby teat canal diameter is increased:

immediately after removal of the milking machine and 4 h or more after milking. It is important to note that no measurements of teat canal diameter were taken in the study of McDonald (1975) between milking and 2 h from milking or between 2 h from milking and 4 h, giving us little precision on the exact time of minimal teat canal diameter. Based on these results, McDonald (1975) identified the period following milking as a risk period for acquisition of IMI and recommended to keep cows standing during this period, whereas no recommendations were made for the second period of increased teat canal diameter, 2 to 4 h following milking. Similarly, Shultz and Bright (1983) found 2 periods of increased penetrability of the papillary duct: up to 30 min following milking and again 2 to 4 h following milking. Similar to McDonald (1975), more precise measurements between 30 and 120 min or between 120 min and 4 h were not collected and, again, recommendations were made regarding the first period following milking but not for the second period of increased penetrability. The same pattern has been observed with different measures of teat recovery following milking in a more recent study (Neijenhuis et al., 2001).

It was hypothesized by McDonald (1975) that larger diameter of the teat canal during the second period of increased susceptibility is caused by hydrostatic pressure created by the accumulation of milk within the teat and gland sinuses. Results from our study clearly suggest that this second period of increased teat canal susceptibility is of tremendous importance in regards to the acquisition of new IMI compared with the period immediately following milking. One possible explanation for the lower odds of IMI for cows lying down for the first time in the period immediately following milking compared with the ones lying down for the first time >90 min later is the presence of freshly applied teat dip on their teat end. Post-milking teat dipping was part of the routine milking procedures on the 6 farms studied and its efficacy against new environmental IMI is well accepted. It is probable that the presence of the freshly applied teat dip would provide a better protection from IMI during this first period of increased teat canal susceptibility.

In our study, the relatively low number of new IMI prevented us from conducting pathogen-specific analyses and one could easily question the inclusion of new *Corynebacterium* spp. IMI or even question the exclusion of new *Staphylococcus aureus* IMI. Most (66%) of the new IMI considered in our analyses were caused by CNS, which are, in fact, a group composed of a wide variety of different *Staphylococci*. Although the exact epidemiology of each different member of this group probably varies quite a lot, it has been shown that CNS IMI incidence is determined in part by practices related

to management of the environment of the cow (Dufour et al., 2008); prevention of this type of IMI could, therefore, be achieved by practices preventing contact of the teat end with the cow's environment such as preventing the cows from lying down after milking. Similarly, most *Streptococcus* and coliform IMI, which represent 11% of the new IMI considered in our analyses, could theoretically be affected by such a management practice. Finally, 23% of the new IMI were caused by *Corynebacterium* spp., for which a definite scheme for infection of the quarters has not been clearly demonstrated. As a sensitivity analysis evaluating the effect of the inclusion or exclusion of a specific pathogen, estimates of the effect of post-milking standing time on IMI occurrence were obtained (data not shown) for a model where all types of new IMI were considered and also for a model where new IMI caused by *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Corynebacterium* spp. were excluded. Exactly the same trend of increasing odds of acquiring a new IMI as post-milking standing time increased past 60 min were seen in both models with similar strength of association; the joint *P*-values for post-milking standing time categories were 0.08 and 0.04, respectively, for the all-IMI models and the IMI models without *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Corynebacterium* spp.

As previously mentioned, some cross-sectional studies have observed that dairy producers making an effort to promote longer post-milking standing time had greater odds of having a low SCC or a lower incidence of clinical mastitis (Peeler et al., 2000; Barnouin et al., 2004). Associations revealed by such cross-sectional studies must, however, be appreciated with great care because they highlight only an association present at one point in time without any knowledge of the time order of occurrence between the dependant and independent variables, rendering the differentiation of cause and effect very difficult. Also, the association observed could, in fact, be a proxy for an unmeasured underlying process such as dairy producer knowledge or attitude regarding udder health. Our decision to use a longitudinal cohort study and to measure individual cow standing time (rather than a herd-level measurement) to determine the association of post-milking standing time with the incidence of IMI (rather than IMI prevalence) was motivated by the need to clearly establish the role of post-milking standing time as a possible risk factor for acquisition of IMI and is clearly one of the strengths of this study.

CONCLUSIONS

Our results suggest that the provision of feed around milking time (between 30 min before and 60 min after)

results in the longest post-milking standing times in tie stall-housed cows. Provision of feed outside this time frame (either before or after) has differential effects on post-milking standing times and is likely influenced by the willingness of the cows to initiate a new meal after milking or by the anticipation of the delivery of fresh feed. Although we have demonstrated that post-milking standing time can be managed by around 15 min by providing fresh feed at different moments around milking, the results obtained in the post-milking standing time-IMI incidence analyses render the use of such a feeding strategy in tie stall systems clearly unrealistic as an IMI prevention strategy. The cows lying down for the first time 90 min or more after milking are the ones with higher odds of acquiring a new IMI; therefore, future research on teat canal susceptibility should focus on this particular time period. Future research should also focus on the relationship between post-milking standing time and IMI incidence in other housing systems where the motivation to remain standing following milking may be different.

ACKNOWLEDGMENTS

We thank Bianca Kitts and Mike MacLean of the University of Guelph, Kemptville Campus (Ontario, Canada) for their technical help through the data collection and summarizing periods. This research was financed in part by Natural Science and Engineering Research Council of Canada; Alberta Milk; Dairy Farmers of New Brunswick, Nova Scotia, Ontario, and Prince Edward Island; Novalait Inc.; Dairy Farmers of Canada; Canadian Dairy Network; Agriculture and Agri-Food Canada; Public Health Agency of Canada; Technology Prince Edward Island Inc.; Université de Montréal; and the University of Prince Edward Island, through collaboration with the Canadian Bovine Mastitis Research Network.

REFERENCES

- Barkema, H. W., J. D. Van Der Ploeg, Y. N. Schukken, T. J. G. M. Lam, G. Benedictus, and A. Brand. 1999. Management style and its association with bulk milk somatic cell count and incidence rate of clinical mastitis. *J. Dairy Sci.* 82:1655–1663.
- Barnouin, J., M. Chassagne, S. Bazin, and D. Boichard. 2004. Management practices from questionnaire surveys in herds with very low somatic cell score through a national mastitis program in France. *J. Dairy Sci.* 87:3989–3999.
- Broll, S., S. Glaser, and L. Kreienbrock. 2002. Calculating sample size bounds for logistic regression. *Prev. Vet. Med.* 54:105–111.
- Canadian Council on Animal Care. 1993. Guide to the Care and Use of Experimental Animals. Vol. 1. E. D. Olfert, B. M. Cross, and A. A. McWilliam, ed. CCAC, Ottawa, Ontario, Canada.
- Cook, N. B., and D. Reinemann. 2007. A toolbox for assessing cow, udder and teat hygiene. Pages 31–43 in *Proceedings of the Annual Meeting of the National Mastitis Council*. National Mastitis Council Inc., Verona, WI.

- DeVries, T. J., K. A. Beauchemin, F. Dohme, and K. S. Schwartzkopf-Genswein. 2009. Repeated ruminal acidosis challenges in lactating dairy cows at high and low risk for developing acidosis: Feeding, ruminating, and lying behavior. *J. Dairy Sci.* 92:5067–5078.
- DeVries, T. J., K. A. Beauchemin, and M. A. G. von Keyserlingk. 2007. Dietary forage concentration affects the feed sorting behavior of lactating dairy cows. *J. Dairy Sci.* 90:5572–5579.
- DeVries, T. J., and M. A. G. von Keyserlingk. 2005. Time of feed delivery affects the feeding and lying patterns of dairy cows. *J. Dairy Sci.* 88:625–631.
- DeVries, T. J., M. A. G. von Keyserlingk, and K. A. Beauchemin. 2005. Frequency of feed delivery affects the behavior of lactating dairy cows. *J. Dairy Sci.* 88:3553–3562.
- Dohoo, I., W. Martin, and H. Stryhn. 2003. *Veterinary Epidemiologic Research*. AVC Inc., Charlottetown, Prince Edward Island, Canada.
- Dufour, S., I. Dohoo, T. DeVries, and D. Scholl. 2008. Improving mastitis control programs through identification of risk factors related to the incidence of subclinical intramammary infections in mastitis control—From science to practice. Pages 211–218 of the *Proceedings of the 2008 International Conference on Mastitis Control*. Wageningen Academic Publishers, Wageningen, the Netherlands.
- Dufour, S., H. W. Barkema, L. DesCôteaux, T. J. DeVries, I. R. Dohoo, K. Reyher, J.-P. Roy, and D. T. Scholl. 2010. Development and validation of a bilingual questionnaire for measuring udder health related management practices on dairy farms. *Prev. Vet. Med.* (accepted.)
- Fregonesi, J. A., C. B. Tucker, and D. M. Weary. 2007. Overstocking reduces lying time in dairy cows. *J. Dairy Sci.* 90:3349–3354.
- Harmon, R. J., R. J. Eberhart, D. E. Jasper, and B. E. Langlois. 1990. *Microbiological Procedures for the Diagnosis of Bovine Udder Infection*. National Mastitis Council, Arlington, VA.
- Hogan, J. S., R. N. Gonzalez, S. C. Harmon, S. P. Nickerson, J. W. Oliver, J. W. Pankey, and K. L. Smith. 1999. *Laboratory Handbook on Bovine Mastitis*. Revised ed. NMC Inc., Madison, WI.
- Johansson, B., I. Redbo, and K. Svennersten-Sjaunja. 1999. Effect of feeding before, during and after milking on dairy cow behaviour and the hormone cortisol. *Anim. Sci.* 68:597–604.
- McDonald, J. S. 1975. Radiographic method for anatomic study of the teat canal: Changes between milking periods. *Am. J. Vet. Res.* 36:1241–1242.
- Neijenhuis, F., H. W. Barkema, H. Hogeveen, and J. Noordhuizen. 2000. Classification and longitudinal examination of callused teat ends in dairy cows. *J. Dairy Sci.* 83:2795–2804.
- Neijenhuis, F., G. H. Klungel, and H. Hogeveen. 2001. Recovery of cow teats after milking as determined by ultrasonographic scanning. *J. Dairy Sci.* 84:2599–2606.
- Olde Riekerink, R. G. M., H. W. Barkema, D. F. Kelton, and D. T. Scholl. 2008. Incidence rate of clinical mastitis on Canadian dairy farms. *J. Dairy Sci.* 91:1366–1377.
- Peeler, E. J., M. J. Green, J. L. Fitzpatrick, K. L. Morgan, and L. E. Green. 2000. Risk factors associated with clinical mastitis in low somatic cell count British dairy herds. *J. Dairy Sci.* 83:2464–2472.
- Reyher, K., S. Dufour, D. Du Tremblay, H. Barkema, L. DesCôteaux, T. J. DeVries, I. R. Dohoo, J.-P. Roy, and D. T. Scholl. 2009. The National Cohort of Dairy Farms—A research platform for mastitis management, planning and control in Canada. Pages 134–135 in *Proceedings of the 48th Annual Meeting of the National Mastitis Council*, Charlotte, NC. National Mastitis Council Inc., Madison, WI.
- Schultz, T. A. 1985. Feeding affects post-milking cow activities. *Calif. Agric.* 39:29–30.
- Schultze, W. D., and S. C. Bright. 1983. Changes in penetrability of bovine papillary duct to endotoxin after milking. *Am. J. Vet. Res.* 44:2373–2375.
- Shook, G. E. 1993. Genetic improvement of mastitis through selection on somatic cell count. *Vet. Clin. North Am. Food Anim. Pract.* 9:563–581.
- Tyler, J. W., L. K. Fox, S. M. Parish, J. Swain, D. L. Johnson, H. A. Grasseschi, and R. Gant. 1997. Effect of feed availability on post-milking standing time in dairy cows. *J. Dairy Res.* 64:617–620.