Effects of metritis on stall use and social behavior at the lying stall

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ABSTRACT

Metritis in dairy cows has been associated with changes in behavior at the feed bunk, but little is known about the effects on behavior at the lying stall. The aim of this study was to investigate stall use by primiparous dairy cows diagnosed with metritis, specifically time spent in the stall, social interactions at the stall, and lying-related behaviors. After parturition, primiparous cows were housed in a mixed-parity pen with a constant group size of 20. Cows had access to 12 electronic feed bins, 2 electronic water bins, and 24 lying stalls. Four cameras installed above the experimental pen allowed for observation of cows in the feeding and lying area. Every 3 d after parturition, cows were examined for metritis by evaluation of the visual appearance and olfactory character of vaginal discharge. Cows diagnosed with metritis ($n = 16$) were compared with healthy individuals (i.e., cows without metritis or other clinical disease, $n = 16$). Healthy individuals were selected based on data availability, body weight, and calving date and, based on these criteria, paired with metritic cows. Video of the 3 d before diagnosis (d -3 to d -1) in the metritic animals (and video from the corresponding days in milk for paired healthy cows) were used to measure behavior. Behaviors assessed included those in the stall (lying, perching, and standing fully in the stall), social behaviors (when a cow either displaced or was displaced by another cow; i.e., actor and reactor replacements), and lying-related behaviors (including visits when the cow entered and left the stall without lying down, aborted lying events when behaviors indicative of the onset of a lying bout were not followed by the cow lying down, and latency to lie down, defined as the time between an aborted lying event and the first lying bout). Cows with metritis spent more time standing fully in the stall on all 3 d, resulting in more time spent standing on d -2 and -1 . Cows with metritis tended to have more aborted lying events on d

-2 , and significantly more on d -1 . Cows with metritis tended to be replaced more often at the lying stall on d -3 and tended to have a longer latency to lie down on d -2 . We observed no differences between health groups in the number of actor replacements or the number of visits to the stall. In summary, cows with metritis spent more time standing fully in the lying stall and had more aborted lying events. These results suggest that primiparous cows with metritis may be identified by altered behavior at the lying stall.

Key words: sickness, pain, disease, welfare

INTRODUCTION

Reduced feeding, decreased social interactions, and increased resting are associated with the onset of sickness in many mammals (Dantzer and Kelley, 2007). In dairy cows, feeding behaviors and social behaviors at the feed bunk in particular have been explored, and some of these behaviors have shown promise as early indicators of disease. Compared with healthy cows, ill cows consume less DM (Huzzey et al., 2007; Fogsgaard et al., 2015), spend less time feeding (González et al., 2008; Goldhawk et al., 2009), engage in fewer competitive interactions at the feeder (Huzzey et al., 2007), and avoid the feed bunk at peak feeding times when competitive interactions are most common (Sepúlveda-Varas et al., 2016).

Avoidance of social interactions by ill cows may also be evident in contexts other than feeding. For example, cows that became ill with either mastitis or metritis in the days following calving increased use of an area visually separating them from the remainder of the herd (Proudfoot et al., 2014). Freestalls do not provide visual separation, but as partitions at the feed bunk reduce competitive social interactions during feeding (DeVries and von Keyserlingk, 2006; Hetti Arachchige et al., 2014), lying stall partitions that separate animals from one another may also offer a protective environment and therefore may be more attractive when animals are ill.

Dairy cows alter lying behavior in response to sickness (Sepúlveda-Varas et al., 2014; Itle et al., 2015) and pain (Mølgaard et al., 2012). Multiple studies have

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reported shorter lying times in cows with clinical mastitis compared with healthy animals, suggesting that cows may be avoiding painful pressure on the inflamed udder (Siivonen et al., 2011; Fogsgaard et al., 2012, 2015). Previous work has found that cows experiencing painful conditions (e.g., dystocia and mastitis) lie down more often (i.e., have more lying bouts; Proudfoot et al., 2009; de Boyer des Roches et al., 2017), but other work has shown that cows with metritis lie down less often in the days before diagnosis (Neave et al., 2018). However, changes in lying time in response to disease are not always observed; for instance, cows with metritis, an infectious disease associated with visceral pain (Stojkov et al., 2015), showed no difference in lying times compared with healthy cows (Neave et al., 2018), despite showing other signs of malaise (e.g., reduced feed intake; Huzzey et al., 2007; Schirmann et al., 2016) and reduced activity (measured by neck-mounted accelerometer; Liboreiro et al., 2015). In summary, sickness and pain can both lead to behavioral changes, but these changes may differ depending upon the cause, making it difficult to predict how cows that experience both sickness and pain will respond. For example, metritis is frequently associated with a febrile response (Sheldon et al., 2006) that might be expected to increase lying time, as ill animals typically rest for prolonged periods (Hart, 1988); however, metritis is also associated with visceral pain (Stojkov et al., 2015), possibly resulting in avoidance of lying down or hindering the lying down movement.

To our knowledge, no research has investigated if cows alter their behavior in response to malaise or pain at the lying stall independently from lying times. Thus, the objective of our study was to investigate stall use, including times spent at the stall, social interactions, and lying-related behaviors, by primiparous cows diagnosed with metritis. We hypothesized that cows with metritis would increase stall usage, specifically standing in the stall, engage in fewer antagonistic interactions and show more aborted lying events in the days before diagnosis.

MATERIALS AND METHODS

Cows in our study were part of a larger project on metritis investigating the effects of parity on changes in behavior (Neave et al., 2017), sickness behavior in the days before diagnosis (Neave et al., 2018), and treatment with meloxicam (Lomb et al., 2018). The study was conducted from July 2013 to October 2014 at the University of British Columbia's Dairy Education and Research Centre in Agassiz (BC, Canada). All animals were cared for according to the Canadian Council on Animal Care (2009) and procedures were approved

by the University of British Columbia's Animal Care Committee (Protocols A10-0163 and A14-0040).

Housing and Management

During the study, behaviors and the health status of all animals in the herd ($n = 337$ Holstein cows) were monitored from approximately 3 wk before to 3 wk after calving. By the end of the study period, data for 105 primiparous cows had been collected.

Within 24 h after parturition, all cows were moved into a mixed-parity postpartum pen where they remained for a minimum of 21 d. Whenever a freshly calved cow was introduced, the cow with the highest DIM (DIM at removal ranged from 21 to 37, with a mean of 30) was removed to maintain a stocking density of 20. On a single occasion in spring 2014, where a longer lag occurred between calving events, 8 cows with high DIM were replaced with pregnant filler cows to maintain stocking density. Feed and water were provided from 12 electronic feed bins and 2 electronic water bins, respectively (Insentec, Marknesse, Holland; validated by Chapinal et al., 2007). The lying area consisted of 24 lying stalls, equipped with mats (Pasture Mat, Promat Inc., Woodstock, ON, Canada) covered with 5 cm of sand. The stalls were 120 cm wide (center to center) and 260 cm long, with the brisket board placed 170 cm from the inside of the back curb. The neck rail was positioned at 115 cm above the bedded surface, 165 cm as measured from the horizontal axis, and 200 cm as measured from the diagonal axis to the inside of the back curb. Stalls were raked clean twice daily when the cows were brought up for milking, and fresh sand was added on Monday and Friday every week of the study. Concrete floors in both alleys (i.e., feed alley and the alley between lying stalls) and crossover alleys were covered with vulcanized rubber mats (Gummiwerk KRAIBURG Elastik, Tittmoning, Germany).

Cows were milked twice daily at approximately 0700 and 1700 h. At approximately 0800 and 1600 h, cows were provided fresh feed formulated to meet the NRC (2001) nutrient requirements. Feed samples were taken once weekly, pooled into monthly samples, and analyzed. The postpartum cow TMR included 26% corn silage, 13% grass silage, 7% alfalfa hay, 4% grass hay, and 50% grain concentrate mash ($DM = 50.35 \pm 2.5\%$, $CP = 18.3 \pm 0.58\% DM$, $ADF = 18.1 \pm 0.71\% DM$, $NDF = 28.5 \pm 1.2\% DM$, and $NE_L = 1.72 \pm 0.014$ Mcal/kg; described in full by Neave et al., 2017).

Diagnosis of Disease

Based on the methodology described by Huzzey et al. (2007), cows were screened for metritis on every

third day after calving, beginning at 3 DIM and lasting until 21 DIM. For this purpose, animals were moved into a sorting area immediately after morning milking (between 0800 and 1000 h) and restrained using headlocks. Metritis diagnosis was based on the visual appearance (color, consistency, presence of pus) and olfactory sensation (no odor/foul smell) of vaginal discharge using a 5-point scoring system, from 0 to 4 (described by Huzzey et al., 2007); cows with a clear, cloudy or bloody mucus and no smell were diagnosed with a healthy uterus (score 0 and 1), whereas cows with foul-smelling discharge that either contained pus or was of watery, brownish character were diagnosed with metritis (scores 2–4). On the morning of diagnosis (between 0800 and 1000 h), cows with metritis were enrolled in the study described by Lomb et al. (2018), where cows were randomly treated with the nonsteroidal anti-inflammatory drug meloxicam or a placebo in addition to an antimicrobial.

Retained placenta was diagnosed when fetal membranes were still attached 24 h after calving. Other clinical diseases (i.e., mastitis, ketosis, displacement of abomasum) were diagnosed based on standard farm protocols or by the herd veterinarian. Calving ease was categorized as unassisted, easy pull (i.e., 1 person assisted) or dystocia (i.e., hard pull with more than 1 person assisting or malpresentation of the calf). Beginning the first day after calving, the rectal body temperature of each animal was taken daily between 0800 and 1200 h using a digital thermometer (Nexcare Rapid Digital Thermometer, 3M, St. Paul, MN) that was inserted 10 cm into the rectum and pressed against the lateral wall. All cows were weighed twice within the first 3 d after calving, with values averaged to provide a mean BW. Cows were categorized as healthy if they were not diagnosed with any clinical disease, including dystocia, and did not have a body temperature $>39.5^{\circ}\text{C}$ more than once during the 21-d observation period.

Study Animals

All primiparous cows diagnosed with a metritis score 4 were considered for participation in the study. Out of the 26 initially eligible cows, 10 were excluded due to incomplete video records of the 3 d before metritis diagnosis. The remaining 16 cows were diagnosed with metritis on either DIM 6 ($n = 11$), 9 ($n = 3$), or 12 ($n = 2$), had an average BW of 574 ± 65.3 kg, and an average daily milk yield over the first 21 DIM postpartum of 24 ± 4.9 kg/d.

From the healthy primiparous cows ($n = 42$), 16 animals were selected as match pairs to the metritic animals. The identification of the match pairs took place by first, excluding any animals with missing

video data ($n = 12$). Second, of the remaining animals, healthy cows were matched with metritic cows based on BW and, when possible, calving date. Lastly, when more than 1 cow had a similar BW and calving date, selection was random. The identified 16 healthy cows had an average BW of 603 ± 51.6 kg and an average daily milk yield over the first 21 DIM of 24 ± 4.2 kg/d. With the exception of 1 healthy cow that had a metritis score of 0, the remaining 15 healthy animals all had a metritis score of 1.

Behavioral Measures

All behaviors were assessed by video. Four cameras (CCTV camera, model WVCW504SP, Panasonic, Osaka, Japan) were mounted 6 m above the experimental pen, 2 above the feed alley and 2 above the lying stalls. Cameras were connected to a digital video surveillance system (GeoVision, GeoVision Inc., Corona, CA). Cows were marked on their back and sides with alphanumeric symbols using hair dye to allow for individual identification.

Behaviors of metritic cows were assessed on the 3 d before diagnosis (d -3 to -1), before any treatment was given to the participating cows (Lomb et al., 2018). The 3-d window for behavioral observations was chosen given that cows were scored as clinically healthy (non-metritic) during the health check 3 d before metritis diagnosis. For healthy animals, the 3 corresponding DIM to the metritic pair were analyzed. To account for the time of diagnosis, days were adjusted to start at 1000 h; for example, d -1 included the 24-h interval from 1000 h on the day before diagnosis to 1000 h on the day of diagnosis.

Behaviors were scored by 3 trained observers, and agreement was tested using Cohen's kappa (Cohen, 1960). Observers reached excellent inter- and intra-observer agreement ($\kappa > 0.75$; Kaufman and Rosenthal, 2009) for all reported behaviors. One of these observers, blind to health status of the animal, scored all aborted lying events.

Position and Time Spent in the Stall. The overall time each cow spent at the lying stall, as well as the positions she adopted in the stall, was measured using 5-min scan samples. The specific positions recorded were lying, with abdomen touching the ground; perching, with 2 front feet in the stall; and standing fully in stall, with all 4 feet in the stall. If at the moment of the scan the cow was changing her position, the position she adopted at the end of the movement was recorded.

The duration of each behavior in minutes per day was estimated by multiplication of the number of scans by 5 (the length of the sampling interval, described and validated for standing behavior in cattle by Chen

et al., 2016). Reported are lying time (min/d), time spent perching (min/d), time standing fully in the stall (min/d), time spent standing in the stall (min/d), and total time at the stall (min/d). The total time standing in the stall was defined as the sum of time spent perching and standing fully in the stall. The total time at the stall was defined as the sum of lying and standing time in either position in the stall.

Social Behavior. Social behaviors at the lying stalls were observed by continuous assessment of the video. Social interactions at lying stalls included actor replacement, when the focal cow used physical contact (e.g., head butt, push) to force another cow out of the stall, and thereafter either entered the stall or remained outside the stall; and reactor replacement, when the focal cow was forced out of the stall by physical contact (e.g., head butt, push) from another cow. The position of the reactor cow (lying, perching, or standing fully in the stall) at the time of displacement was also noted for each event. The number of times she was replaced out of either a lying or standing position was calculated in relation to the time she spent in that position (i.e., number of position replacements/h spent in this position).

Lying-Associated Behaviors. Continuous observations were also used to assess behaviors related to lying down. To lie down in the stall, a cow must enter with all 4 feet. Once she had entered a stall, we recorded either a (1) visit, when the cow left without lying down, or (2) an aborted lying event, when the cow displayed behaviors associated with lying down but did not do so. An aborted lying event was recorded when the cow (1) completed at least 1 sweeping head movement (Österman and Redbo, 2001) that crossed her centerline while lowering her head to the ground (Tucker and Weary, 2004); (2) completed a minimum of 2 steps or leg movements toward the center of her body (underneath) while her head was lowered to the ground; or (3) lowered herself from a standing position to her carpal joints but then moved back into the standing position without lying down.

When cows actually completed the lying down movement, the latency to lie down was recorded as the time from the first aborted lying event until she successfully lay down; intervals that precluded stall use (e.g., milking, sand delivery) were not included. We report the daily number of visits to the lying stalls, the daily number of aborted lying events, and the average latency to lie down (min/lying bout).

Statistical Analyses

All statistical analyses were performed using SAS (Version 9.4; SAS Institute Inc., Cary, NC), with cow

as experimental unit and behaviors summarized per cow and day. Variables were screened by health group using PROC UNIVARIATE. Four variables (standing fully in the stall, reactor replacements when standing fully in the stall, aborted lying events, and average latency to lie down) were transformed using the $\log_{10}$ -transformation to normalize distributions for further analyses.

Behavioral differences between healthy and metritic cows were analyzed using PROC MIXED in SAS. The initial model included BW (kg), DIM at diagnosis (diagnosis day; 3 levels: 6, 9, and 12), and health status (2 levels: healthy vs. metritis) as between-cow factors, and day relative to diagnosis (observation day; 3 levels: d -3, d -2 and d -1) as a within-cow factor. The interaction of health status with observation day was also offered to the model; this interaction was evident (with $P \leq 0.10$) for 2 of the outcome variables (standing fully in the stall and reactor replacements). Hence, all analyses were performed by observation day, with BW, diagnosis day, and health status as covariates in the model.

Results for normally distributed variables are presented as least squares means and standard errors. Results for variables that were $\log_{10}$ -transformed are reported as back-transformed geometric means and 95% confidence interval on the original scale. The significance level was set at $P < 0.05$ and tendencies are reported when $P \leq 0.10$.

We observed a low frequency of replacements out of a lying position [metritis = 0 (0–0.33); healthy = 0.17 (0–0.33) no./60 min; median (Q1 – Q3)]. Therefore, treatment differences were not tested for this measure. Differences in lying time and daily number of lying bouts were previously reported by Neave et al. (2018), where cows with metritis had fewer, but longer lying bouts; these measures are only reported here when necessary to supplement outcome measures of interest.

RESULTS

On d -2, cows diagnosed with metritis tended to spend more time standing in the stall than healthy cows ($F_{1,28} = 3.62$, $P = 0.07$); the difference was more pronounced on d -1 ($F_{1,28} = 8.92$, $P < 0.01$; Table 1). This difference was driven by an increase in standing fully in the stall by metritic animals (d -3: $F_{1,28} = 4.31$, $P < 0.05$; d -2: $F_{1,28} = 15.9$, $P < 0.001$; d -1: $F_{1,28} = 21.77$, $P < 0.001$; Figure 1) on all 3 d before diagnosis. We observed no differences between the health groups in time spent perching and the total time spent at the stall.

We found no difference in the number of actor replacements between healthy and metritic cows, but noted a

Table 1. Stall positions, social behaviors, and lying behaviors of healthy (H, n = 16) and metritic (M, n = 16) primiparous cows in the 3 d before diagnosis¹

Item	d -3			d -2			d -1		
	H	M	SE	H	M	SE	H	M	SE
Position at stall (min/d)									
Perching	230	221	20.6	257	261	22.4	213	256	25.5
Lying ²	628 ± 85	613 ± 142.9	NA	596 ± 135.7	559 ± 106.8	NA	534 ± 124.8	517 ± 118.6	NA
Standing in stall ³	261	310	20.5	284*	352*	24.9	231 ^a	348 ^b	27.3
Total time in stall ⁴	899	913	31.7	891	901	24.6	780	850	31.3
Social interactions (no./d)									
Actor replacements	2.1	2.4	0.5	1.9	1.7	0.8	3.4	1.4	1.2
Reactor replacements	1.9*	3.3*	0.5	3.4	3.5	0.8	3.0	2.4	0.7
Reactor position when replaced (no./60 min)									
Standing fully in stall ⁵	1.1	1.4	NA	1.3	1.4	NA	1.2	1.3	NA
	(0.9-1.4)	(1.1-1.8)		(1-1.7)	(1.1-1.9)		(0.9-1.5)	(1.0-1.8)	
Perching	0.5	0.6	0.1	0.6	0.6	0.1	0.6	0.3	0.1
Lying down behaviors									
Visits to the stall (no./d)	7.6 ^{ab}	6.5	1.5	7.0	7.7	1.6	4.4	5.4	1.1
Aborted lying events ⁵ (no./d)	3.4	4.9	NA	3.2*	5.4*	NA	2.2 ^a	4.2 ^b	NA
	(2.2-5.3)	(3.2-7.6)		(2-4.9)	(3.5-8.3)		(1.5-3.2)	(2.9-6.1)	
Average latency to lie down ⁵ (min/lying bout)	3.0	5.3	NA	2.7*	6*	NA	2.9	4.7	NA
	(1.7-5.3)	(3-9.1)		(1.4-5.3)	(3.1-11.9)		(1.6-5.2)	(2.6-8.4)	
Lying bouts ² (no./d)	15 ± 5.2	14.7 ± 4.3	NA	15.1 ± 4.8	14.4 ± 3.8	NA	13.6 ± 4.5	13.2 ± 3.2	NA

^{a,b}Different superscripts within a row and day indicate $P \leq 0.05$ between health categories.¹Results are reported as LSM ± SE for normally distributed variables. Non-normally distributed variables were log₁₀-transformed for the analysis and reported as back-transformed geometric means and 95% CI. NA = not applicable.²Differences in these outcome measures were previously reported by Neave et al. (2018); these measures are only reported here (mean ± SD) to supplement outcome measures of interest and were not analyzed statistically.³Sum of time a cow spent perching and standing fully in the stall.⁴Sum of time a cow spent perching, standing fully and lying in the stall.⁵Not-normally distributed variables; data are reported as back-transformed geometric means and 95% CI.* $P \leq 0.1$ between health categories.

Figure 1. Standing fully in the stall in metritic (M, $n = 16$) versus healthy (H, $n = 16$) primiparous dairy cows in the 3 d before diagnosis. Whiskers show $1.5 \times$ interquartile range, boxes show interquartile range, the white line shows the median, the open diamonds show the mean, and the filled triangles show outliers (in the healthy cow group, the outlier was to the same cow on all 3 d and in the metritic cow group the outliers are from 2 different cows).

tendency on d -3 for healthy cows to be replaced less often ($F_{1,28} = 3.59$, $P = 0.07$). Cows were displaced out of the 2 standing positions (perching and standing fully in the stall) at similar frequencies.

Cows with metritis tended to have a higher number of aborted lying events compared with their healthy counterparts on d -2 ($F_{1,28} = 2.99$, $P = 0.09$) and had more aborted lying events on d -1 ($F_{1,28} = 5.9$, $P < 0.05$). Cows with metritis also tended to have longer average latencies to lie down than healthy cows on d -2 ($F_{1,28} = 2.84$, $P = 0.1$). Healthy and metritic cows did not differ in the number of visits to the lying stall.

DISCUSSION

As predicted, primiparous cows diagnosed with metritis spent more time standing in the stall. This difference became more pronounced over the 3-d observation period, possibly due to the progression of metritis. The difference in standing time in the stall was driven by prolonged standing with all 4 feet in the stall by the metritic cows. Previous work has shown that standing in the stall with all 4 feet is dependent on stall design (Tucker and Weary, 2004; Tucker et al., 2005); for example, when the neck rail is placed in a less restrictive position, at 190 cm distance to the rear curb, cows increase the time they spend standing fully in the stall (Fregonesi et al., 2009). Interestingly, Fregonesi

et al. (2009) reported that the early-lactation healthy cows used in their study only spent approximately 36 min/d engaged in this behavior. In contrast, the sick primiparous cows in our study stood more than twice as long with 4 feet in the stall, despite the presence of a more restrictively placed neck rail (165 cm from the rear curb, horizontally measured). This difference is consistent with the idea that cows with metritis are more highly motivated to perform this behavior.

One response to sickness in social animals is self-isolation (Hart 1988). Proudfoot et al. (2014) reported that, in contrast to healthy cows, freshly calved dairy cows diagnosed with mastitis or metritis spent more time behind a plywood board that provided a visual barrier from the herd. Similarly, sick calves were found to lie down further away from pen mates than healthy ones (Cramer and Stanton, 2015; Cramer et al., 2016). Cows in our study were not able to visually or physically separate themselves from the group, but spending time standing in the stall may have provided some form of protection. DeVries and von Keyserlingk (2006) and Jensen et al. (2008) provide evidence that partitions at the feeder reduce competitive behaviors in cows and calves, respectively. We suggest that the increased time spent standing fully in the stall can be viewed as a type of self-isolation sickness behavior. However, given the influence of stall design on this behavior, future work is needed to investigate how stall dimensions and cow

size influence this change in behavior. In addition, we may have gained a better understanding of when stall standing behaviors change in relation to clinical symptoms with a daily examination of vaginal discharge as opposed to the 3-d interval.

Ill animals typically increase resting time, likely to conserve energy and support the systemic response to infection (Hart, 1988). However, as reported in our previous paper (Neave et al., 2018), we noted no differences in lying time between metritic and healthy cows. We hypothesized that this might be a consequence of the sick cows being hesitant to lie down, possibly in response to visceral pain (Stojkov et al., 2015), as longer standing times have also been reported after liver biopsy (Mølgaard et al., 2012) and ruminal acidosis (DeVries et al., 2009). We therefore predicted that metritic cows would show more behaviors indicating a reluctance to lie down, as measured by aborted lying events. Similar abnormal lying down movements as described in our study have been used by others to measure stall comfort (Krohn and Munksgaard, 1993; Tucker and Weary, 2004; Dippel et al., 2009), as well as responses to different milking regimens (Österman and Redbo, 2001) and oligofructose overload (Niss et al., 2009). We suggest that pain due to lying down may explain the health group differences in the current study, especially because these differences became more pronounced with time. Visceral inflammatory pain can be referred to other viscera or somatic areas (viscero-visceral or viscero-somatic pain; Gebhart and Bielefeldt, 2016). Visceral pain and viscero-visceral pain likely contributed to increased back arch during transrectal uterine palpation in metritic versus healthy cows (Stojkov et al., 2015). In the current study, referred pain to the viscera may have resulted in pain during lying movements that put tension or pressure on the viscera. Visceral pain may have also led to somatic hyperalgesia (e.g., Wessellmann and Lai, 1997), making skin areas sensitive to touch during lying movements and when lying. We encourage future studies to include measures of hyperalgesia in dairy cows with uterine infection.

The observed reluctance to lie down, measured by aborted lying events, may explain why primiparous cows with metritis had shorter lying times than expected given their illness (Neave et al., 2018). This may also have contributed to the increase in standing fully in the stall by animals with metritis; despite no differences in latencies to lie down, metritic cows may have initially searched for a suitable lying space but then remained standing in the stall.

Avoidance of competitive interactions at the feed bunk has been reported in dairy cows before diagnosis with metritis and mastitis (Huzzey et al., 2007; Sepúlveda-Varas et al., 2016), providing the basis for

our prediction that metritic cows would take part in fewer agonistic interactions at the lying stalls. However, we found little evidence of a difference between health groups. The high number of actor replacements in ill cows in our study may be explained by increased motivation of these animals to access the lying stalls, resulting in increased time spent fully standing in the stall.

The combined number of actor and reactor displacements from the lying stalls in the current study was higher than that reported by others using similar stocking densities (Fregonesi et al., 2007; Val-Laillet et al., 2008; Winckler et al., 2015). These previous studies used mid-lactation, healthy cows housed in stable groups. The higher replacement rate in our study may be explained by the frequent regrouping due to the dynamic group structure. In addition, we only observed primiparous cows that were likely at greater risk of being replaced than multiparous animals as these animals likely had a lower rank within the group hierarchy. Wierenga (1990) reported that younger cows were replaced more often at the feeder and at the lying stalls. Interestingly, standing in the stall in either position (perching or standing fully in the stall) did not seem to provide protection for the cows with metritis, given that they were displaced similarly often as healthy cows. A growing body of literature describes behavioral changes before diagnosis of clinical disease in dairy cows, but much of this focuses on DMI and feeding behaviors. With the development of technology that allows for real-time tracking of cow location within the pen (Meunier et al., 2017; Wolfger et al., 2017) and real-time detection of lying behavior (Porto et al., 2013), it is now also feasible to use behavioral changes at the lying stalls.

CONCLUSIONS

Metritic cows spent more time standing in the stall. Behavioral differences may have been part of a general response to sickness or may be specifically associated with pain, as suggested by an increased number of aborted lying events. Our findings suggest that increased times standing in the stall may be helpful in identifying sick dairy cows.

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