Cardiac responses to palpation per rectum in lactating and nonlactating dairy cows

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ABSTRACT

Interest in the monitoring of heart rate variability (HRV) has increased recently, as it gives more detailed and immediate information about the level of stress than traditional behavioral or hypothalamus-pituitary-adrenal measures. In this study, we evaluated heart rate (HR) and parasympathetic HRV parameters to monitor cardiac stress responses to palpation per rectum (PPR) in lactating (LACT; n = 11) and nonlactating (NLACT; n = 12) dairy cows. Heart rate and HRV were recorded from 40 min before PPR until 120 min after it was completed. Heart rate, the root mean square of successive differences (RMSSD), and the high-frequency component (HF) of HRV were analyzed by examining 5-min time windows. To compare cardiac responses to PPR between groups, changes in HR and HRV parameters were calculated as area under the curve (AUC) for LACT and NLACT cows. An immediate increase in HR was detected during PPR in both LACT (+21.4 ± 2.4 beats/min) and NLACT cows (+20.6 ± 2.3 beats/min); however, no differences were found between groups on the basis of parameters of AUC. The increase in HR in both groups along with a parallel decrease in RMSSD (LACT cows: −5.2 ± 0.4 ms; NLACT cows: −5.1 ± 0.4 ms) and HF (LACT cows: −10.1 ± 0.8 nu (where nu = normalized units); NLACT cows: −16.9 ± 1.2 nu) during PPR indicate an increase in the sympathetic, and a decrease in the parasympathetic tone of the autonomic nervous system. The increase in RMSSD (LACT cows: +7.3 ± 0.7 ms; NL cows: +17.8 ± 2.2 ms) and in HF (LACT cows: +24.3 ± 2.6 nu; NLACT cows: +32.7 ± 3.5 nu) immediately after PPR indicated a rapid increase in parasympathetic activity, which decreased under the baseline values 10 min following PPR. The amplitude and the maximum RMSSD and HF values were greater in NLACT cows than in LACT animals, suggesting a higher short-term cardiac responsiveness of NLACT cows. However, the magnitude and the duration of the stress response were greater in LACT cows, as indicated by the analysis of AUC parameters (area under the HRV response curve and time to return to baseline). Cow response to the PPR was more prominent in parasympathetic HRV measures than in HR. Based on our results, the effect of PPR on the cows’ cardiac stress responses may have an impact on animal welfare on dairy farms, and investigating the effect of lactation on the cardiac stress reactions could prove useful in modeling bovine stress sensitivity. Further research is needed to find out whether the differences due to lactation are physiological or management related.

Key words: heart rate, heart rate variability, palpation per rectum, dairy cow

INTRODUCTION

On commercial dairy farms, stressors are prevalent in a wide variety and intensities. Transrectal examination [e.g., palpation of the uterus per rectum (PPR)] is a frequent procedure performed by veterinarians (Baille et al., 2005). Palpation of the uterus per rectum is used for the detection of postpartum uterine diseases (LeBlanc et al., 2002; Sheldon et al., 2006), during AI, and as the most common method, it is used for early pregnancy diagnosis in dairy cattle (Youngquist, 1997; Romano et al., 2007). The procedure is usually quickly done by skilled veterinarians, yet it can last up to 5 to 10 min for inexperienced students in training. Palpation of the uterus per rectum is a nontraumatic
procedure; however, it can result in physiological stress reactions (Nakao et al., 1994; Waiblinger et al., 2004; Cingi et al., 2012).

It has been demonstrated that merely the presence of humans can cause discomfort in dairy cows (Hagen et al., 2004). In farm animals, an increasing number of studies have used a combination of physiological and behavioral measures for the assessment of pain as a stressor. Behavioral responses are useful for the qualitative description of the sensory experiences and nervous processes (Mellor et al., 2000; Pilz et al., 2012); however, interpretation of behavior is often observer dependent (Mason and Mendl, 1993).

Measures of hypothalamus-pituitary-adrenal axis activity (e.g., plasma cortisol concentrations) have extensively been used to evaluate pain in cattle (Stafford and Mellor, 2011; Coetzee, 2013). However, measures of the activity of the autonomic nervous system (ANS) may have advantages over measuring hypothalamus-pituitary-adrenal activity when investigating responses to acute stress, as they provide more immediate and detailed information (Stewart et al., 2010; Ledowski et al., 2012; reviewed by Kovács et al., 2014).

Different methods exist for evaluating ANS function. Heart rate (HR) mainly reflects the activity of the sympathetic nervous system (SNS; Hopster et al., 1995; Sgoifo et al., 1999); however, it is difficult to draw conclusions about underlying control mechanisms related to ANS activity from HR measurements exclusively (Sayers, 1973; Hainsworth, 1995; Malliani, 1995). Measuring HR variability [HRV; i.e., the short-term fluctuations in the variability of successive cardiac interbeat intervals (IBI)] is the most effective and least invasive method for the assessment of ANS regulatory activity in dairy cattle (von Borell et al., 2007; Kovács et al., 2014). An increasing number of studies have used HRV indices as indicators for the response of the ANS to stress. Using the IBI and calculating parameters in time and frequency domains, it is possible to measure the prevailing balance between SNS and parasympathetic nervous system (PNS) activity (von Borell et al., 2007). To our knowledge, only a few studies exist that describe HRV changes in response to pain in adult farm animals [horse (Rietmann et al., 2004); sheep (Stubsjöen et al., 2009)].

Acute stress activates the SNS; however, defining HRV as a measure of SNS activity remains a subject of debate (Aksehrod et al., 1981; Houle and Billman, 1999). It is generally agreed that the root mean square of successive differences (RMSSD) between the consecutive IBI is the primary time domain measure of HRV that represents vagal regulatory activity (von Borell et al., 2007). The high-frequency component (HF) of HRV is strongly associated with the baroreceptor input, which varies with the breathing cycle and also reflects the changes in PNS tone (Aksehrod et al., 1981; Malliani, 1995). Both parameters have been studied in dairy cattle welfare research (Kovács et al., 2014). Reduced vagal tone was found in cows subjected to waiting after parlor milking with nonvoluntary exit (Kovács et al., 2013) or during milking in a novel milking environment (Sutherland et al., 2012), and in calves exposed to external stress or pathological loads (Mohr et al., 2002).

In cattle, short-term cardiac responses to pain-evoking procedures have been investigated only in calves. In these experiments, animals were subjected to hot-iron disbudding (Stewart et al., 2008) or surgical castration (Stewart et al., 2010) to test the efficiency of local anesthesia. Authors have reported contradictory findings. In the case of castration without local anesthesia, an increase in PNS parameters was found after surgery, whereas in their earlier study, RMSSD and HF decreased following disbudding without local anesthesia, suggesting that the procedure was painful. Those authors suggested that this was due to the varying nature of the pain (somatic vs. visceral), which resulted in higher vagal response to castration, as the PNS is highly involved in carrying noxious impulses from the testes.

In the present work, we monitored HR and HRV parameters in lactating and nonlactating dairy cows before, during, and following PPR in a field study. As severe pain is characterized by a propensity to evoke strong autonomic responses (Ness and Gebhart, 1990) and the rectum is considered as a visceral organ and has only autonomic innervation, we expected that ANS indices of HRV would be useful to study stress reactions of cows. We hypothesized lower cardiac responses to PPR in lactating cows than in nonlactating ones, as this procedure is usually done during the postpartum period, whereas nonlactating animals are not exposed to PPR during the late prepartum period.

**MATERIALS AND METHODS**

**Animals and Housing**

A total of 23 multiparous [11 lactating (LACT) and 12 nonlactating (NLACT)], clinically healthy Holstein-Friesian cows were selected from a large-scale herd (2,000 lactating cows) of Boly Co. (Cspötelek, Hungary). The LACT and NLACT group averages were similar in age (mean ± SD; 3.8 ± 1.3 vs. 3.5 ± 1.1 yr), parity (3.1 ± 0.9 vs. 2.6 ± 0.7 lactations), and BCS (3.1 ± 0.6; vs. 3.3 ± 0.8). Cows were housed in modern freestall barns bedded with sand. The barns were equipped with self-locking headlocks (head gates and headrails; Arntjen Germany GmbH, Rastede, Ger-
many) along the full length of the feeding area. Cows locked themselves in place when putting their heads in the stanchions to eat, after being milked. It was a routine practice on the farm that regular checkups and treatments were carried out while the animals remained restrained and standing after feeding. Such a feeding and restraint system has been used for years. Total mixed ration was fed once per day at 800 h and animals had free access to water. Lactating cows (mean ± SD; DIM = 112 ± 21.5 d; daily milk yield = 47.8 ± 8.6 kg) were milked 3 times per day in a 72-stall rotary milking parlor (BouMatic Xcalibur 360; BouMatic, Madison, WI). Nonlactating animals were pregnant and at least 10 d after drying off and 20 d before expected calving.

**Experimental Design**

The experiment was carried out in November 2013, during a 4-d period. Three cows from each group were involved each day. The Polar Equine RS800 CX mobile recording system (Polar Electro Oy, Kempele, Finland) was used, with 2 integrated electrodes and a specific transmitter. After soaking the body surface under the electrodes with tap water, transmitters and the 2 electrodes were positioned on the thoracic region, as advised by von Borell et al. (2007) in their review. Electrode sites were covered with ample ultrasound transmission gel (AquaUltra Blue; MedGel Medical, Barcelona, Spain) without shaving the skin. The devices were fitted while the animals were standing restrained in the headlocks after feeding, 18 h before the start of recordings to allow enough time to acclimatization to wearing the equipment (Mohr et al., 2002; Gygax et al., 2008).

Heart rate recordings started on the next day, approximately 10 min after the animals had finished feeding, having returned from the morning milking. After a baseline period of 40 min, the PPR was performed. For the accuracy of HRV analysis (see Analysis of HRV section), the examination lasted 5 min, which was longer than what is usual in typical practice. The PPR was done with care. After removing part of the feces, examiners tried to localize the reproductive organs (the cervix and horns of the uterus, and the ovaries), the arteria uterina media, and in the case of pregnant cows, the approachable cotyledons. The examiners were unfamiliar to the cows. Heart rate recordings continued for 120 min after the PPR had finished. To avoid the influence of moving on HR, the experimental animals remained standing throughout the whole length of the recording period. To avoid the influence of separation during this time, neighboring cows on each side were also left restrained. All other cows were released at the time the PPR were finished. Any other kinds of disturbance (e.g., sudden noise and presence of people) or any unnecessary contact with animals throughout the whole experimental period was avoided.

**Analysis of HRV**

The IBI recorded from 40 min before the examination to 120 min afterward were used in the analysis. The HRV analysis was performed using Kubios 2.1 HRV software (Niskanen et al., 2004). A custom filter was applied to correct for any artifacts. Interbeat intervals differing from the previous IBI by more than 30% were identified as artifacts. An average error rate of 5% was accepted for analysis. In addition, a visual inspection of the corrected data was performed to edit out any artifacts still existing. Only one 5-min time window from the baseline measurements and four 5-min time windows from the post-PPR recordings were excluded, due to artifacts. Due to equipment failure, IBI data between 100 and 120 min after PPR was not recorded for 1 cow.

For the interpolation of IBI time series before analysis using fast Fourier transformation (FFT), segments of 512 IBI were examined (ESC-NASPE Task Force, 1996; von Borell et al., 2007) and then HRV was analyzed in the following periods: (1) in the 40 min before PPR (PrePPR); (2) during the 5-min of PPR, and (3) during the 120 min following PPR (PostPPR). As baseline, the mean values in the 15 min before the examination were used. The analyzed time domain measures were mean HR and RMSSD. As a frequency domain parameter, HF was chosen, presented in normalized units, calculated by FFT. Recommendations of von Borell et al. (2007) were fulfilled by setting the limits of the spectral components (low frequency = 0.05–0.20 Hz and HF = 0.20–0.58 Hz). This frequency band width for the HF power was also used by earlier reports on dairy cattle for HRV analysis (Mohr et al., 2002; Hagen et al., 2005; Kovács et al., 2013).

**Statistical Analysis**

Heart rate, RMSSD, and HF were analyzed with a generalized linear model procedure (SPSS 18.0; SPSS Inc., Chicago, IL) with penalized quasi-likelihood. The residuals of the model were inspected graphically for distribution and homogeneity of variances with the Kolmogorov-Smirnov test. Because data were not normally distributed, HR, RMSSD, and HF parameters (as dependent variables) were subjected to logarithmic transformations before analysis. Covariates (parity, condition, and age) were added to the model as fixed
factors. The Bonferroni adjustment was used for post hoc comparisons of HR and HRV values within the groups. A value of $P < 0.05$ was considered significant.

For reducing the number of statistical comparisons between groups during the PostPPR period, changes in HR and HRV parameters were calculated as area under the curve (AUC). The AUC represents both the magnitude and the changes over time of the response (Fekedulegn et al., 2007) and simplifies the statistical analyses by transforming the multivariate data into univariate space, especially when the numbers of repeated measurements are high and a need exists to summarize the information (Watamura et al., 2004). The evaluated parameters included baseline and maximum values of HR, RMSSD, and HF; amplitude (the maximal alteration compared with baseline) of the HR, RMSSD, and HF response; and long-term measures of cardiac responses to the PPR (AUC response and time to return to baseline). The area under the response curve (AUCresp) was determined for the period of time to return to baseline using a method described by Lay et al. (1996):

$$\text{AUC}\text{resp} = \Sigma[(C_n + C_{n+1})/2 \times h - \text{baseline}],$$

where $C$ is the cardiac parameter (HR, RMSSD, or HF) at a given time point, $h$ is the time in hours between the 2 $C$ values, and baseline is the mean value over the final 15 min of recorded HR and HRV data before PPR. The AUC 40 min before PPR was also calculated for both LACT and NLACT groups (AUCPRE). As there was no response above baseline values of either HR or HRV parameters to measure, the area was simply calculated as follows:

$$\text{AUC}\text{pre} = \Sigma[(C_n + C_{n+1})/2 \times h].$$

The AUCpre and AUCPOST parameters are presented in absolute values. The nonparametric Friedman test was used for the comparison of AUC parameters between groups because of substantial non-normality in several parameters (evaluated by Levene’s test). The level of significance was set at $P < 0.05$.

**RESULTS**

Changes in HR showed a similar pattern in both LACT and NLACT cows throughout the experiment (Figure 1a). In the PrePPR period, HR did not change significantly in either group. During PPR, HR increased ($P < 0.001$, in both groups), and in the first 5 min after PPR it decreased and then returned back to the normal (baseline) values.

Although baseline and maximum HR were significantly higher ($P < 0.001$, in both cases) in LACT than NLACT group, the amplitude of HR did not differ between groups (Table 1). The AUC analysis did not detect any effect of the lactation either in the PrePPR or in the PostPPR period. After returning to baseline, HR was relatively stable in both studied groups, with an average slightly below the physiological level in LACT and slightly above that in NLACT cows (Figure 1a).

Root mean square of successive differences—similarly to HR—did not change considerably in the PrePPR period in either group (Figure 1b). Baseline RMSSD was lower in the LACT group, compared with the NLACT group ($P < 0.001$); AUCPRE was similar in both groups (Table 1). During PPR, RMSSD decreased in both LACT and NLACT cows ($P < 0.001$ and $P < 0.01$, respectively), reflecting the sudden decrease in the PNS activity. The RMSSD in the first 5 min of PostPPR highly exceeded the baseline in both groups ($P < 0.001$). The rate of the increase was, on average, 24.4 and 41.7% in the LACT and NLACT groups, respectively. The maximum and amplitude of RMSSD were lower in the LACT group, compared with the NLACT group ($P < 0.001$ and $P < 0.01$, respectively). For LACT cows, RMSSD took longer to return to baseline ($P < 0.001$) and AUCRESP was also greater in LACT cows ($P < 0.01$; Table 1).

Similarly to RMSSD, baseline HF was lower in LACT than NLACT cows ($P < 0.001$). During PPR, HF decreased in both groups ($P < 0.001$; Figure 1c). The rate of decline was, on average, 28.5% in LACT and 38.1% in NLACT cows, respectively. Area under the curve parameters of HF also differed between groups. A higher maximum in the 5 min following PPR ($P < 0.001$) and higher amplitude ($P < 0.05$) was found in NLACT cows, whereas LACT cows showed a greater AUCRESP ($P < 0.001$) and longer time to return to baseline ($P < 0.001$) measured in HF (Table 1).

**DISCUSSION**

Baseline HR differences between LACT and NLACT cows are consistent with that found earlier (Mohr et al., 2002), yet those authors did not find the difference (83 vs. 74 beats/min) relevant. We suggest that this difference can primarily be attributed to the increased energy demands of lactation, as higher milk production is correlated with higher heart rate (Weiss et al., 2004). Such effect of milk production on ANS activity is also reflected in baseline RMSSD and HF differences between LACT and NLACT cows. The findings of Hagen et al. (2005) suggest that other factors, such as housing, management, and breed, could also be influential, as
Figure 1. Changes in (a) heart rate (HR; beats/min), (b) the root mean square of successive differences (RMSSD; ms), and (c) the high-frequency component [HF; nu (where nu = normalized units)] of heart rate variability (HRV) in nonlactating (▲; n = 11) and lactating cows (●: n = 12) before, during, and after palpation per rectum (PPR). For significant differences between groups for cardiac response, parameters are calculated as area under the curve (AUC). Mean baseline HR, RMSSD, and HF values are represented by the horizontal lines. Values are means ± SEM.
they measured an average 6.6 ms of baseline RMSSD and 16.2 normalized units for baseline HF in their study on lactating Brown Swiss and Fleckvieh cows. During the baseline period, HR, RMSSD, and HF as well as AUC regarding any of the cardiac parameters did not change significantly in any of the groups, which suggests that restraint and the proximity of examiners did not eventuate considerable stress for the animals studied.

The elevation in HR during PPR is a good indicator of acute stress (Hopster et al., 1995; Sgoifo et al., 1999). The amplitude of the HR response did not differ between groups (average of 16 beats/min in both groups). Mialon et al. (2012) reported a similar rate of increase (80.9 vs. 96.9 beats/min) in adult cattle, in response to rumenocentesis (a rumen fluid collection procedure) without local anesthesia; however, baseline data were recorded after the restraint. When cows were restrained, they found no evidence to indicate that rumenocentesis was more stressful than handling the animals.

Waiblinger et al. (2004) found an average 7 beats/min increment in the HR of dairy cows during PPR performed by people unfamiliar to the animals. The animals were regularly examined as subjects of veterinary education, which might explain why the HR response was lower than what we observed. The difference could also be due to the short acclimatization period in their study, as the electrodes were attached only 35 min before PPR; therefore, baseline values (10–20 min before PPR) were likely to have been elevated. In the abovementioned studies, animals were fixed in restraining cages or insemination stalls during the experiment and they were isolated.

Baseline recordings may be biased in isolation or in surroundings that the animal associates with previous unpleasant experiences due to the triggering of moderate stress reactions, even without any manipulation (Boissy and Bouissou, 1994). In our study, cows were examined in their regular feeding place and were not isolated. Despite the confinement and the presence of experimenters during and after PPR, animals were relatively calm. We could conclude that the observed cardiac reactions are due to stress associated with the procedure of PPR.

Investigating short-term stress responses during castration without local anesthesia in bull calves,

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1Descriptive statistics are based on mean ± SD of nontransformed data.
2AUC PRE = area under the curve during 40 min before PPR; baseline = the last 15 min before PPR; amplitude of response = the maximal alteration compared with baseline; AUC RESP = area under the response curve calculated for the period of time to return to baseline.
3nu = normalized units.
4*P < 0.05; **P < 0.01; ***P < 0.001 [statistical significances for HR, root mean square of successive differences (RMSSD), and HF response parameters are based on logarithmically transformed data. P-values for differences between groups are based on results from the Friedman test].
The use of HRV parameters provides a noninvasive means to assess ANS responses to acute stress in adult cattle. Lactating and nonlactating cows differ in their basal cardiac activity measured in standing posture. Lactating cows exhibited lower short-term cardiac responsiveness to PPR than NLACT animals, whereas in terms of magnitude and duration, cardiac responses mirrored by PNS indices of HRV were more intensive.
in LACT cows than NLACT ones. Further research is needed to clarify these differences in ANS activity between NLACT and LACT animals. As PPR caused significant stress in both LACT and NLACT cows, the length and frequency of the transrectal examination should be as minimal as possible, especially during training.

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