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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 (Baillie 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

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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 (Stubsjøen et al., 2009)].

Acute stress activates the SNS; however, defining HRV as a measure of SNS activity remains a subject of debate (Akselrod et al., 1985; 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 (Akselrod 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 Bóly Co. (Csípőtelek, 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-