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Influence of postoperative pain and use of NSAID on heart rate variability of dairy cows

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This Research Communication describes the effect of post-operative pain and non-steroidal anti-inflammatory drug (NSAID) treatment on heart rate variability (HRV) of dairy cows. Postoperative pain in farm animals is often left untreated, and HRV could be a promising tool for assessing pain. The aim of this study was to assess if postoperative state after subcutaneous surgery affects HRV in dairy cows and to determine whether this could be modulated by NSAID. Nine cows were inserted with an implantable electrocardiograph logger. Cows were divided into the NSAID treatment group and the control group. The cows in the NSAID group had higher HRV than the control group, indicating a higher sympathetic activity in control animals, most likely due to untreated post-operative pain. Besides the ethical need for treating pain in production animals, ongoing pain has an adverse effect on animal productivity. Thus post-operative pain alleviation is recommended.

Keywords: Dairy cattle, heart rate variability, pain, nonsteroidal anti-inflammatory drug.

In most cases involving farm animals, intraoperative pain during veterinary procedures is treated properly, but treatment for postoperative pain is seldom given (Walker et al. 2011). Behaviours such as restlessness, foot stamping and changes in lying behaviour are used for assessing farm animal pain, but also physiological parameters, such as stress hormones, are assessed (Viñuela-Fernández et al. 2007). However, both behavioural and traditional physiological approaches have their limitations in sensitivity and reliability and new methods for effective pain assessment are needed. Heart rate variability (HRV) can be used as an indicator of the sympathovagal balance of the autonomous nervous system (Task Force, 1996) and HRV is a promising tool for pain assessment in farm animals (calves: Stewart *et al.* 2008, 2010; ewes: Stubsjøen et al. 2009). However, results of HRV measurements are inconsistent: pain has been shown either to increase sympathetic (e.g. Stewart et al. 2008; Stubsjøen et al. 2009) or vagal (e.g. Stewart et al. 2010) activity. Thus there is a need for greater understanding of the underlying mechanism of HRV response, especially for different types of pain (e.g. somatic and visceral).

In cattle, studies on pain and HRV are mainly conducted with disbudded or castrated calves (Stewart et al. 2008, 2010) rather than with adult animals. The aim of the study was to assess if postoperative somatic pain after small-scale surgery and postoperative nonsteroidal anti-inflammatory drug (NSAID) treatment affect HRV in dairy cows. This experiment was a part of a larger study focusing on the effect of stress on HRV in dairy cows measured with implantable technology, and approved by the National Animal Experiment Board (Permit Number: ESHL-2008-08892/Ym-23).

Materials and methods

The study was conducted at the Natural Resources Institute Finland research barn in Maaninka, Finland. The experimental animals were four pregnant heifers and seven dry cows (six Holstein-Friesian and five Ayrshire). The animals were tethered in peat-bedded rubber-matted tie stalls (width 120 cm, length 180 cm). They were fed daily with

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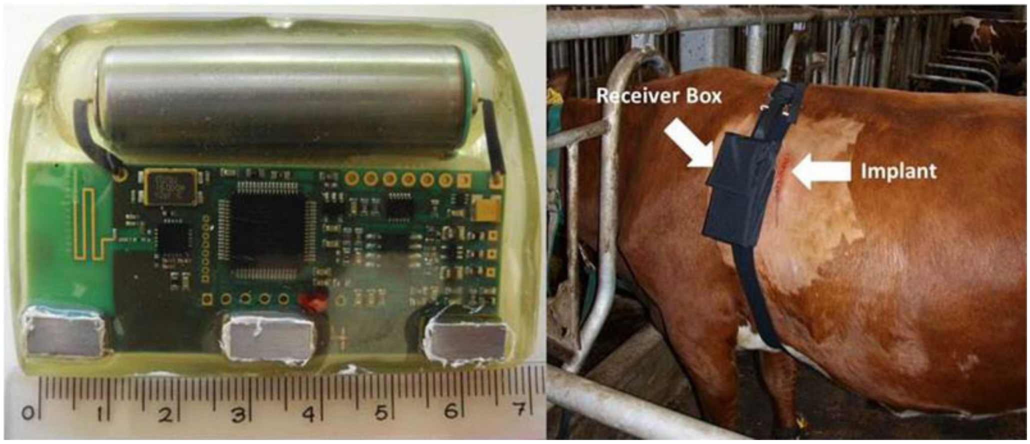

Fig. 1. Implant for ECG measurements and implant and receiver box after operating on the cow.

0·3 kg of barley concentrate (energy content 13·2 MJ ME/kgDM), 0·2 kg commercial mineral for dry cows (Tunnu-Namino, Hankkija Oy, Finland), and 32 kg (multiparous cows) or 36 kg (heifers) grass silage (10·5 MJ ME/kgDM).

Implantable loggers (Bjarnason et al. 2013) were used to measure the electrocardiograph (ECG) of the experimental animals (Fig. 1) with a sample rate of 341·3 Hz. The system contained a 50 g (70 mm × 45 mm × 17 mm) implant placed on the left side of the animal into a pocket made between the skin and subcutaneous tissue posterior to the scapula, and a receiver box attached to the side of the cow with an elastic girth. The animals were operated about one month (±3 d) prior to the expected parturition date under sedation (0·018 mg/kg xylazine hydrochloride 20 mg/ml intravenously) and local anaesthesia (20–35 ml lidocaine hydrochloride 20 mg/ml subcutaneously). The animals received three-day antibiotic treatment (20 000 IU/kg penicillin benzathine and penicillin procaine 300 000 IU/ml intramuscularly) post-operatively.

Heifers and cows were randomly divided into the treatment (1 heifer + 5 cows) and the control (3 heifers + 2 cows) groups. On the operation day the treatment group received intravenous NSAID treatment (3 mg/kg ketoprofen 100 mg/ml). An oral NSAID treatment (4 mg/kg ketoprofen 160 mg/g) mixed in a small amount of concentrates continued for four consecutive days (days 1–4) after the operation day. The control group did not receive any NSAID treatment, but a small amount of concentrates as a control treatment on the days 1–4. Oral ketoprofen has been effectively used previously e.g. with acute mastitis in dairy cows (Banting et al. 2008).

ECG of the experimental animals was measured and behaviour was video recorded during days 1–4 using cameras (Axis Q1755-E and Axis 215) positioned to face downwards to the tie-stalls. The body and head postures and rumination of the animals were recorded continuously twice daily, 05·00–07·00 and 22·00–24·00. Because the posture of the animal affects the HRV (Frondelius et al. 2015) only the ECG data from continuous lying periods (sternal recumbency while head up) from animals not ruminating were used for the HRV analysis. ECG data from these periods were visually inspected and good quality 5-min segments were selected for further analysis. Data from two control group animals were discarded because of unreadable ECG data due to malfunctioning of the implants.

The ECG data were interpolated to 500 Hz using a geometric interpolation method (Tiusanen et al. 2015) and the location of the S wave (Tiusanen & Pastell, 2016) was used to analyse the normal-to-normal (NN) intervals. We calculated heart rate (HR), the standard deviation of NN intervals (SDNN), root mean square of successive NN interval differences (RMSSD) and pNN50 (Task Force, 1996).

HRV data from nine animals (treatment $n = 6$, control $n = 3$) were used for the analysis including mean daily values of HRV parameters from 94 five-min segments; 58 segments from the treatment group and 36 segments from the control group, 10·4 ± 5·87 (mean ± SD) segments per cow. Linear mixed models were fitted to determine the effect of independent variables – treatment (control *vs.* NSAID), time from the procedure (days 1–4) and the interaction between these two – on HRV parameters (dependent variables) using compound symmetry as a covariance structure and cow as a random effect. The statistical analyses were made with SAS for Windows version 9·2 through SAS Enterprise Guide version 4·3 (SAS Institute Inc., Cary, NC, USA).

Results and discussion

The cows in the treatment group had higher SDNN ($P < 0·05$) and numerically higher RMSSD (non significant) values than the animals in the placebo group (Table 1). There were no differences in HR and pNN50 between two groups. Day did not have any effect on HRV values and there was no interaction between the treatment and the day.

The results indicate that NSAID affects the postoperative HRV of adult dairy cows that had undergone subcutaneous implantation. The control group had a lower vagal tone and a stronger sympathetically controlled response (von Borell

Table 1. Comparison of the linear mixed model estimates of heart rate variability (HRV) variables (mean ± SEM) between NSAID (cows $n = 6$; analysed 5-min HRV segments $n = 58$) and placebo groups (cows $n = 3$; analysed 5-min HRV segments $n = 36$)

	NSAID	Placebo	*P*
HR	61·0 ± 3·84	61·8 ± 5·55	ns
SDNN	56·1 ± 2·16	45·6 ± 3·39	<0·05
RMSSD	60·8 ± 1·96	54·3 ± 3·09	ns
pNN50	6·21 ± 1·58	4·96 ± 2·40	ns

et al. 2007) to the postoperative state, most likely due to untreated pain. Ketoprofen might have also mitigated the inflammatory reaction towards the implant due to NSAIDs anti-inflammatory and anti-pyretic effects (Donalisio et al. 2012).

We did not observe any effect of NSAID treatment on long-term HR. This result is similar to Raekallio et al. (1997), who studied horses for 72 h after arthroscopic surgery. Generally higher sympathetic activity is connected to increase in HR (Von Borell et al. 2007) and lack of pain medication increases HR (Stewart et al. 2008; Stubsjøen et al. 2009). In these studies, however, HR was measured only during the painful procedure or few hours after it. This difference in methods may partly explain lack of difference in HR in our study, since we started measurements of cardiac responses one day after the surgery and measured it throughout the whole four-day post-operative treatment. However, it is also possible that ketoprofen was not a potent enough NSAID to cause differences in HR, as in the study by Newby et al. (2014).

With this small number of animals, our experiment should be regarded as a preliminary study. However, linked with other results, we suggest that HRV may be a sensitive measure for assessing pain in production animals. Stubsjøen et al. (2009), who also used a limited number of animals in their study, found that moderate pain in sheep had an effect on HRV.

This study shows that dairy cows are probably subject to postoperative pain even after small-scale subcutaneous surgery. In production animals postoperative pain is often neglected (Walker et al. 2011). Besides the ethical need for treating pain, ongoing pain may also have an adverse effect on animal productivity, affecting their growth and immune functions (Anil et al. 2005). Thus pain alleviation during and after painful procedures is beneficial for the animal, the producer and the consumer.

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