

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

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### 11 Summary

12 This Research communication describes the effect of post-operative pain and NSAID treatment on  
13 heart rate variability (HRV) of dairy cows. Postoperative pain in farm animals is often left  
14 untreated. HRV could be a promising tool for pain assessment in animals. The aim of this study  
15 was to assess if postoperative state after subcutaneous surgery and NSAID treatment affect HRV in  
16 dairy cows. Nine cows were inserted with an implantable electrocardiograph logger. Cows were  
17 divided into the NSAID treatment group and the control group. The cows in the NSAID group had  
18 higher HRV than the control group, indicating a higher sympathetic activity in control animals,  
19 most likely due to untreated post-operative pain. Besides the ethical need for treating pain in  
20 production animals, ongoing pain has an adverse effect on animal productivity. Thus post-  
21 operative pain alleviation is recommended.

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23 *Key words: Dairy cattle, Heart rate variability, Pain, Nonsteroidal anti-inflammatory drug*

24

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

38

39 In cattle, studies on pain and HRV are mainly conducted with disbudded or castrated calves  
40 (Stewart *et al.* 2008, 2010) rather than with adult animals. The aim of the study was to assess if  
41 postoperative somatic pain after small-scale surgery and postoperative nonsteroidal anti-  
42 inflammatory drug (NSAID) treatment affect HRV in dairy cows. This experiment was a part of a  
43 larger study focusing on the effect of stress on HRV in dairy cows measured with implantable  
44 technology, and approved by the National Animal Experiment Board (Permit Number: ESHL-2008-  
45 08892/Ym-23).

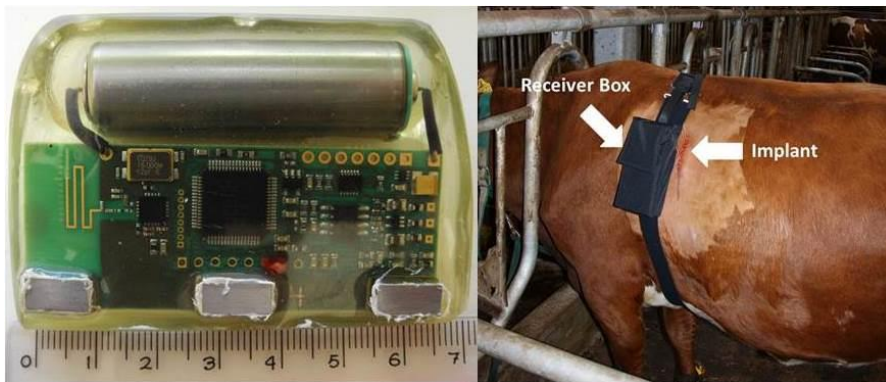
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47 Materials and methods

48 The study was conducted at the Natural Resources Institute Finland research barn in Maaninka,  
49 Finland. The experimental animals were four pregnant heifers and seven dry cows (six Holstein-  
50 Friesian and five Ayrshire). The animals were tethered in peat-bedded rubber-matted tie stalls  
51 (width 120 cm, length 180 cm). They were fed daily with 0.3 kg of barley concentrate (energy  
52 content 13.2 MJ ME/kgDM), 0.2 kg commercial mineral for dry cows (Tunnu-Namino, Hankkija Oy,  
53 Finland), and 32 kg (multiparous cows) or 36 kg (heifers) grass silage (10.5 MJ ME/kgDM).

54  
55 Implantable loggers (Bjarnason *et al.* 2013) were used to measure the electrocardiograph (ECG) of  
56 the experimental animals (Fig. 1) with a sample rate of 341.3 Hz. The system contained a 50 g 70  
57 mm x 45 mm x 17 mm) implant placed on the left side of the animal into a pocket made between  
58 the skin and subcutaneous tissue posterior to the scapula, and a receiver box attached to the side  
59 of the cow with an elastic girth.

60



61  
62 Figure 1. a) Implant for ECG measurements and b) implant and receiver box after operating on the  
63 cow.

64

65 The animals were operated about one month ( $\pm$  3 days) prior to the expected parturition date  
66 under sedation (0.018 mg/kg xylazine hydrochloride 20 mg/ml intravenously) and local anesthesia  
67 (20 – 35 ml lidocaine hydrochloride 20 mg/ml subcutaneously). The animals received three-day  
68 antibiotic treatment (20 000 IU/kg penicillin benzathine and penicillin procaine 300 000 IU/ml  
69 intramuscularly) post-operatively.

70

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

78

79 ECG of the experimental animals was measured and behaviour video recorded during days 1 – 4  
80 using cameras (Axis Q1755-E and Axis 215) positioned to face downwards to the tie-stalls. The  
81 body and head postures and rumination of the animals were recorded continuously twice daily,  
82 05.00 – 07.00 and 22.00 – 24.00. Because the posture of the animal affects the HRV (Frondeus *et*  
83 *al.* 2015) only the ECG data from continuous lying periods (sternal recumbency while head up)  
84 from animals not ruminating were used for the HRV analysis. ECG data from these periods were  
85 visually inspected and good quality five-minute segments were selected for further analysis. Data  
86 from two control group animals were discarded because of unreadable ECG data due to  
87 malfunctioning of the implants.

88

89 The ECG data were interpolated to 500Hz using a geometric interpolation method (Tiusanen *et al.*  
90 2015) and the location of the S wave (Tiusanen & Pastell 2016) was used to analyze the normal-to-  
91 normal (NN) intervals. We calculated heart rate (HR), the standard deviation of NN intervals  
92 (SDNN), root mean square of successive NN interval differences (RMSSD) and pNN50 (Task Force  
93 1996).

94  
95 HRV data from the nine animals (treatment n=6, control n=3) were used for the analysis including  
96 mean daily values of HRV parameters from 94 five-minute segments; 58 segments from the  
97 treatment group and 36 segments from the control group, 10.4±5.87 (mean±SD) segments per  
98 cow. Linear mixed models were fitted to determine the effect of independent variables –  
99 treatment (control vs NSAID), time from the procedure (days 1 – 4) and the interaction between  
100 these two – on HRV parameters (dependent variables) using compound symmetry as a covariance  
101 structure and cow as a random effect. The statistical analyses were made with SAS for Windows  
102 version 9.2 through SAS Enterprise Guide version 4.3 (SAS Institute Inc., Cary, NC, USA).

103

#### 104 Results and discussion

105 The cows in the treatment group had higher SDNN and tended to have higher RMSSD values than  
106 the animals in the placebo group (Table 1). There were no differences in HR and pNN50 between  
107 two groups. The day did not have any effect on HRV values and there was no interaction between  
108 the treatment and the day.

109

110 The results indicate that NSAID affects the postoperative HRV of adult dairy cows that had  
111 undergone subcutaneous implantation. The control group had a lower vagal tone and a stronger  
112 sympathetically controlled response (von Borell *et al.* 2007) to the postoperative state, most likely

Commented [FL1]: Table 1 near here

113 due to untreated pain. Ketoprofen might have also mitigated the inflammatory reaction towards  
114 the implant due to NSAIDs anti-inflammatory and anti-pyretic effects (Donalisio *et al.* 2012).

115

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

125

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

130

131 This study shows that dairy cows are subjected to postoperative pain even after small-scale  
132 subcutaneous surgery. In production animals postoperative pain is often neglected (Walker *et al.*  
133 2011). Besides the ethical need for treating pain, ongoing pain may also have an adverse effect on  
134 animal productivity, affecting their growth and immune functions (Anil *et al.* 2005). Thus pain  
135 alleviation during and after painful procedures is beneficial for the animal, the producer and the  
136 consumer.

137

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203 Table 1. Comparison of the linear mixed model estimates of heart rate variability (HRV) variables  
204 (mean±SEM) between NSAID (cows n=6; analyzed 5-minute HRV segments n=58) and placebo  
205 groups (cows n=3; analyzed 5-minute 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	<0.1
pNN50	6.21±1.58	4.96±2.40	ns