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Pre-test habituation improves the reliability of a handheld test of mechanical nociceptive threshold in dairy cows

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ABSTRACT

Mechanical nociceptive threshold (MNT) testing has been used to investigate aspects of painful states in bovine claws. We investigated a handheld tool, where the applied stimulation force was monitored continuously relative to a pre-encoded based target force. The effect on MNT of two pre-testing habituation procedures was performed in two different experiments comprising a total of 88 sound Holsteins dairy cows kept either inside or outside their home environment. MNT testing was performed using five consecutive mechanical nociceptive stimulations per cow per test at a fixed pre-encoded target rate of 2.1 N/s. The habituation procedure performed in dairy cows kept in their home environment led to lowered intra-individual coefficient of variation of MNT ($P < 0.001$), increased MNT ($P < 0.001$) and decreased the discrepancy between applied and target force during stimulations ($P < 0.001$). Pre-test habituation improved the reliability of the handheld tool when used in dairy cows kept in their home environment.

1. Introduction

The nociceptive threshold can be identified as the intensity of a noxious stimulation which activates the pain sensory system. Persistent injury or intense stimulation may sensitize the pain processing system leading to a decreased nociceptive threshold and/or an exaggerated pain perception to stimuli at the site of the painful lesion and potentially at locations distant from the primary painful lesion (Anderson and Muir, 2005; Basbaum et al., 2009). Nociceptive threshold testing can be used to determine the presence of hyperalgesia, which is clinically characterized by an increased avoidance response, compared to baseline, elicited upon application of a noxious stimulus (Love et al., 2011). In dairy cattle mechanical (Chambers et al., 1994; Whay et al., 1997, 1998; Laven et al., 2008; Tadich et al., 2013) and thermal (laser) (Veissier et al., 2000; Herskin et al., 2003) devices have been used to apply controlled nociceptive stimulations as ramped mechanical forces or constant radiant heat until a behavioural avoidance response occurs. The use of a laser device has the advantage of applying constant nociceptive heat stimulation remote from the subject, but its use in commercial herds is restricted due to safety issues. In bovine orthopaedic research, the application of a ramped mechanical stimulation has usually been performed by pressing a rounded steel pin against the skin of the dorsum of the cannon using an actuator to ensure gradual increase of the force at a constant rate (Ley et al., 1996; Whay et al., 1997, 1998, 2005; Laven et al., 2008; Tadich et al., 2013). The cuff-mounted actuator has been attached to the stimulation limb, which required handling and restraint of the animals, potentially affecting their responses to the stimulations (Veissier et al., 2000). Hence, the use of a handheld tool to measure mechanical nociceptive threshold (MNT) in loose housed animals kept in their home environment is of interest.

Methods to determine MNT by handheld tools have been described in sheep (Stubsjøen et al., 2010), pigs (Janczak et al., 2012; Di Giminiani et al., 2013, 2014) and cattle (Raundal et al., 2014), where large variabilities in the animals' responses to the nociceptive mechanical stimulations are typically reported. Some of these variations are reported to be related to other factors than hyperalgesia, for example fear or stress in the testing situation. As a consequence, inclusion of habituation procedures, aimed to reduce the variability, has been suggested by Raundal et al. (2014). Importantly, the habituation procedure should be aimed only at the test procedure and the initial non-noxious part of the

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ramped stimulation. Learning effects based on repeated ramped stimulations (Le Bars et al., 2001) or habituation or sensitization of the nervous system by repeated noxious stimulations (Bergadano et al., 2009; Mouraux et al., 2012) should be avoided.

It has been recommended to apply a ramped mechanical stimulation at a constant rate (Dundee and Moore, 1960; Janczak et al., 2012). However, when handheld tools are used, the operator's influence may attribute to variation arising from discrepancy between the actual applied force and the ramped target force, given by a predefined constant rate and stimulation time. Operator induced variation is among the main technical challenges of this methodology. Only a few reports exist from MNT studies in large animals using handheld devices, where the rate of stimulation can be controlled (Di Giminiani et al., 2013, 2014), but no studies have been described in cattle.

The objective of the present study was to investigate effects of two different pre-test procedures aiming at habituating dairy cows in the presence of the observer and to the initial tactile phase of the ramped mechanical stimulations on the reliability of a handheld methodology for MNT testing in dairy cows. The effect of habituation on the intra-individual coefficient of variation (CV) of MNT, on MNT and on the discrepancy between the actual applied force and the target force during stimulations, was evaluated.

2. Materials and methods

This study comprised two experiments. Experiment 1 tested the cows outside their home environment. It was carried out at the University of British Columbia (UBC) Dairy Education and Research Centre, Agassiz, Canada, and was approved by the institutional Animal Care Committee at UBC. Experiment 2 tested the cows in their home environment and was carried out at the Danish Cattle Research Centre, Tjele, Denmark. The animal procedures and housing complied with the Danish Animal Experiments Inspectorate, according to the Danish Ministry of Justice Act no. 1306 (November 23rd, 2007), as procedures that do not require specific approval. The habituation and testing procedures as well as visual inspections and lameness scorings (based on Thomsen et al., 2008) of the dairy cows were performed by the same observer (trained veterinarian) in both experiments.

2.1. Experiment 1: testing outside of the home environment

2.1.1. Study design

The purpose of this experiment was to conduct an initial investigation of the effect of a habituation procedure. The experiment was conducted as a matched pair design (Ersbøl et al., 2004) and consisted of a baseline test followed by a retest of all cows. The two test sessions were separated by a period where the treatment cows received a habituation procedure and the matched control cows did not. The experiment was carried out on workdays between 0900 and 1500 h during October and November 2011.

2.1.2. Animals and housing

Experimental cows were selected from the 260 cow research dairy herd at the UBC Dairy Research and education Centre in Agassiz, British Columbia, Canada. Cows were loose housed with sand-bedded cubicles, fed daily at 0700 and 1600 h, with a fresh total mixed ration formulated for high producing dairy cows and milked in a milking parlour at 0800 and 1700 h.

Eighty-five cows met the inclusion criteria: Lactating, non-lame (lameness score below 3, using a 1 (non-lame)–5 (severely lame) point scale, Thomsen et al., 2008) Holsteins, more than 30 days in milk (DIM), and more than 60 days to due date, out of which forty-six cows were chosen from pens closest to the testing area. Experimental cows were blocked in pairs by parity, DIM and state of pregnancy. Within each pair, the cows were randomly allocated to either habituation or control group. Finally pairs were randomly assigned to one of three experimental weeks. Health status was assessed by information from the herdsmen, visual inspection by the observer and rectal temperature (based on the average of two measurements taken by a technician at the end of each nociceptive test session), within 38.0–39.0 °C. Eighteen cows were excluded at the end of the experiment (12 became lame, during the experimental weeks, two were excluded to balance number of habituation and control cows and four cows due to technical difficulties during the familiarization or testing procedures).

2.1.3. Test area and familiarization

The test area (Fig. 1) had concrete flooring which was thinly covered with sand in the mornings of each habituation and test day. Cows were restrained by a head lock on a weigh scale (Pacific Industrial Scale Co. Ltd., Richmond, British Columbia, Canada), that afforded a safe and comfortable space for testing.

Each experimental cow was familiarized during the week prior to the first test week, by walking them through the scale three times. They were restrained for 2 min on the scale during the last two passages. Cows were scored for lameness at the last passage. An extra familiarization session was given on Fridays to cows to be tested the following week. Handlers and the observer wore blue overalls.

2.1.4. Habituation procedure

Cows were baseline tested on Monday mornings and retested on Thursday mornings in the same order. Between the two test sessions, the cows received either one habituation or one control procedure. Since cattle can use the colour of clothes worn by people as a cue to discriminate between humans (Munksgaard et al., 1997), and to increase the difference between the habituation and the control procedure, the observer wore blue overalls for the baseline test and the control procedure, and wore a red coat for the habituation and retest procedures.

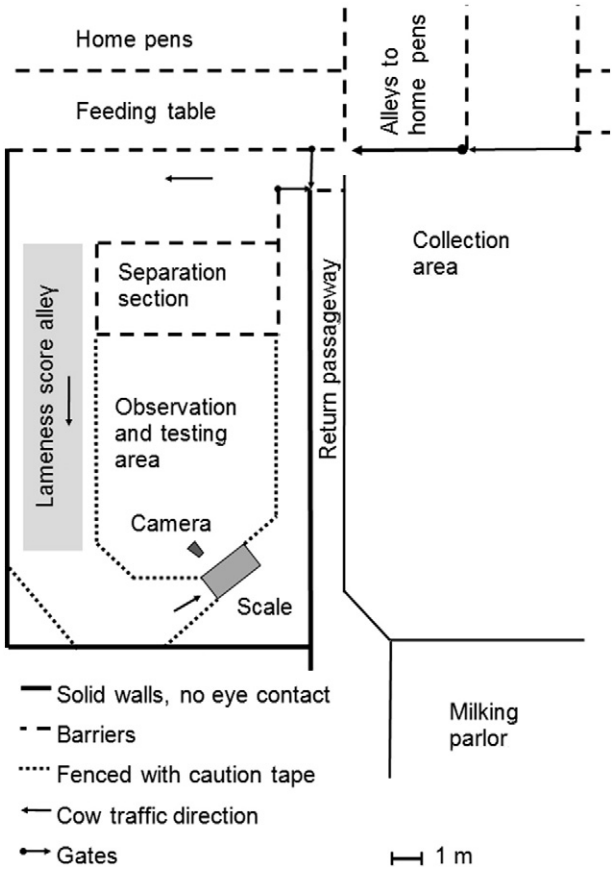

Fig. 1. Outline of testing area in Exp. 1.