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Behavioural and physiological responses to clove oil injected under the horn bud of calves

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ABSTRACT

The aim of this study was to evaluate the acute and longer-term behavioural and physiological responses caused by administering clove oil under the horn bud of calves and compare these with the responses caused by cautery disbudding. Forty female Friesian-cross calves (mean ± SD: 3.5 ± 2.72 d of age) were allocated to one of four treatments (n = 10 calves/treatment): 1) control handling (CON), 2) administration of 0.5 mL of clove oil under each horn bud (CLOV), 3) cautery disbudding (CAUT) or 4) local anaesthetic plus cautery disbudding (LA + CAUT). Lying behaviour was recorded continuously using accelerometers, while other behaviours were recorded from video for the same 2 h periods on 3 days relative to treatment: baseline (day before), D0 (0–24 h after) and D1 (24–48 h after). Pain sensitivity adjacent to the horn bud was measured using pressure algometry, and blood samples were collected to measure complete blood cell counts and serum amyloid A concentrations, before and 48 h after administration of treatments. CAUT calves tended to perform more head shakes than CON and CLOV calves during the first 2 h after treatment (change from baseline, no./2 h: CON: −0.3, CAUT: 4.7, LA + CAUT: 3.0, CLOV: −0.4, SED = 2.33, $P = 0.096$). Compared to baseline, CLOV calves spent less ($P < 0.05$) time head rubbing than LA + CAUT calves on D0, however, on D1 all treatments rubbed their heads less ($P < 0.05$) compared to CON calves. Compared to baseline, CAUT and LA + CAUT calves spent less ($P < 0.05$) time running than CON calves on D0, but the time spent running did not differ between CON and CLOV calves. Mean daily lying times after administration of treatments were greater in CLOV compared to CON and CAUT calves, and CLOV and LA + CAUT calves did not differ (change from baseline (min/24 h): CON: −14.8, CAUT: 25.1, LA + CAUT: 64.2, CLOV: 94.8, SED = 31.77; $P = 0.016$). Overall, all treatments displayed more sensitivity in response to pressure algometry than CON calves (change from baseline (kg of force): CON: −0.3, CAUT: −2.2, LA + CAUT: −1.8, CLOV: −1.5, SED = 0.46; $P = 0.003$). There was no treatment effect on blood constitutes. Our results suggest that injecting clove oil under the horn bud was initially less painful, and in the 48 h after treatment, did not cause more pain than cautery disbudding. Unlike cautery disbudding, injecting clove oil does not involve tissue removal nor is there a risk of thermal damage to the brain. Therefore, clove oil could be an alternative to cautery disbudding, if this method is found to efficaciously prevent horn growth.

1. Introduction

Calves are routinely disbudded to reduce the risk of injury to other animals and stock handlers. Moreover, during transport and lairage, horns can cause bruising and damage to hides of other cattle (Marshall, 1977; Vowles, 1976). Disbudding is most commonly performed in New Zealand using a hot cautery iron (Stafford and Mellor, 2011). However, changes in calf physiology and behaviour in response to cautery disbudding, such as elevated plasma cortisol concentrations, increased ear flicking, head shaking and rubbing (Faulkner and Weary, 2000; Graf and Senn, 1999; Grøndahl-Nielsen et al., 1999), show that this procedure causes acute pain. An increase in the acute phase response and haematological changes associated with inflammation suggest that calves may also experience longer-term post-operative pain after dehorning (Glynn et al., 2013; Sutherland et al., 2013). Moreover, if cautery disbudding is performed poorly (e.g., too much pressure or for too long) then complications such as necrosis and inflammation of the brain can occur (Nation and Calder, 1985). Thus, cautery disbudding negatively affects animal welfare and alternatives to this method should be explored.

Growing worldwide public concern for animal welfare in agricultural systems means that painful husbandry procedures are coming

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under increasing scrutiny. New Zealand's Code of Welfare (National Animal Welfare Advisory Committee, 2005) provides some minimum standards regarding pain mitigation; it encourages wider use of pain relief as well as the development of management systems that would allow for cows with horns. Farmers often have the perception that providing pharmaceutical pain relief for disbudding, such as anaesthesia and/or analgesia, is costly and time consuming (Robbins et al., 2015), as it involves first administrating the pain relief and second performing the disbudding. Preventing horn growth by selecting for polled dairy cattle would be the best solution for calf welfare, but until this solution is more widely accepted by the industry, there remains a need to evaluate practical methods of pain relief or alternative novel disbudding techniques that may eliminate or reduce associated pain.

Clove oil has many well-known medicinal properties (Chaieb et al., 2007) and has been used as a topical analgesic and antiseptic in human dentistry (Markowitz et al., 1992). Furthermore, it has been used as an antibacterial agent in food products (Friedman et al., 2004). These attributes can be linked to clove oil's main component, eugenol. Interestingly, at high concentrations, eugenol also causes cellular necrosis (e.g. 100% eugenol, mucous membrane in the mouth of rats; Kozam and Mantell, 1978) and is cytotoxic (e.g. 73% eugenol, human endothelial and fibroblast cells; Prashar et al., 2006). Recently, clove oil has been shown to cause local cellular necrosis of horn bud cells of calves (Molaei et al., 2014) and goat kids (Molaei et al., 2015), resulting in arrested horn growth. Injecting clove oil to prevent horn growth has the potential advantage over cautery disbudding in that it does not involve tissue removal or the risk of thermal injury to the brain. Both studies suggested that injecting clove oil was not as invasive a procedure as cautery disbudding (Molaei et al., 2015, 2014), however, neither specifically measured the pain response associated with administering clove oil. The acute pain associated with cautery disbudding persists for at least 4 h, but calves may still experience pain for at least 44 h after disbudding (Heinrich et al., 2010). Currently there is no literature describing the acute or longer-term behavioural or physiological responses to administering clove oil under the horn bud of calves. Therefore, the aim of this study was to evaluate the acute and longer-term pain response caused by administering clove oil under the horn bud of calves and to compare this with the response caused by cautery disbudding. It was predicted that injecting clove oil as a method of horn growth prevention would cause less acute behavioural and physiological pain responses than cautery disbudding.

2. Material and methods

2.1. Animals, housing and experimental design

This study was conducted from March to May 2017 on a commercial dairy cow farm in the Waikato region, New Zealand. All procedures involving animals were approved by the AgResearch Animal Ethics Committee (protocol No. 14116) under the New Zealand Animal Welfare Act 1999.

Forty Friesian-cross dairy heifer calves (mean ± SD: 36.0 ± 4.36 kg; 3.5 ± 2.72 d of age) were used in the study. All calves were allowed to suckle from the dam, and then were separated (maximum time with dam approx. 12 h). Calves were transported to the farm's calf rearing facility, and fed additional colostrum until full, according to standard practice on this farm. The calf rearing facility had solid dirt floors covered in wood chip and solid walls on three sides of the building; the front of the facility was open, and gates open to the outside served as the forth wall of the building. Calves were kept in holding pens prior to being moved to the experimental pens. Groups of four calves were held in experimental pens (3 m × 6 m, 4.5 m^2/calf) constructed from wooden panels that allowed auditory, visual, olfactory and some tactile contact between animals in adjoining pens. Each pen had a plastic trough attached to a gate containing *ad libitum* pellets (HiPro Sweet Calf Stage 1, James & Son (NZ) Pty. Ltd., Wellington, NZ), and a large round trough providing *ad libitum* access to water. Calves were fed 2.5 L of colostrum twice a day at 0800 and 1630 for the first 4 d after birth. Thereafter, the equivalent amount of milk was provided using a five-teat milk feeder (Stallion Plastic Ltd, Palmerston North, NZ) which was removed after each feeding.

Temperature and humidity was measured throughout the trial using Lascar data loggers (EL-USB-2-LCD +, Lascar Electronics Ltd. Salisbury, UK), which were attached at calf head height within each pen. The average temperature and humidity inside the facility over the entire study period was 16.6 °C (range: 5.1 °C–27.6 °C) and 83.3% (range: 46.7%–98.2%), respectively.

Calves were held in a holding pen for approximately 4 days before being enrolled and moved to the experimental pen on the first day (baseline) of the study. Calves were weighed and marked with paint (Tell-tail paint, FIL New Zealand, Mount Maunganui, New Zealand) across the back and sides to allow for individual identification during video analysis. Hair within the horn bud area was clipped to facilitate locating the horn bud prior to disbudding. Calves were fitted with an accelerometer data logger (HOBO Pendant G, 64k, Onset Computer Corporation, Bourne, MA, USA) and blood samples were taken from the jugular vein.

On the following day (treatment day, D0), calves were restrained in a calf crush for measurement of baseline mechanical nociceptive thresholds (MNT) and then the calves were randomly allocated to one of four treatments (n = 10 calves/treatment). Treatments included: 1) control handling (CON), 2) cautery disbudding (CAUT), 3) local anaesthetic plus cautery disbudding (LA + CAUT) and 4) administration of clove oil under the horn bud (CLOV). For CON, calves were restrained, a hand was used to massage the horn bud region, but calves were not disbudded. For CAUT, an electric cautery iron ("Quality" electric debudder, 230 V, 190 W; Lister GmbH, Lüdenscheid, Germany) was used to remove calf horn buds. For LA + CAUT, the same disbudding method used for CAUT was used, except that 5 min prior to disbudding, these calves were injected with 3 mL of local anaesthetic (2% lignocaine hydrochloride; Lopain, Ethical Agents Ltd., Auckland, NZ) into the cornual nerve. For CLOV, clove oil was injected subcutaneously (0.5 mL/horn bud) under each horn bud using an 18 gauge ½ inch needle (Fig. 1A). The needle was inserted from the side of the horn bud proximal to the midline and directed under the bud towards the base of the outer portion of the ear. The needle was stopped and clove oil was injected once the tip of the needle was situated under the centre of the horn bud (Fig. 1B). Four calves, one calf per treatment, were then moved into each experimental pen. Once all the treatments had been administered, the calves were left undisturbed for 48 h except for normal farm procedures (e.g. feeding).

Forty-eight hours after administration of treatments, calves were restrained, MNT around the horn was measured using pressure algometry, blood samples were taken and the accelerometers were removed. CAUT, LA + CAUT and CLOV calves were injected subcutaneously with meloxicam (0.2 mg/kg; Metacam, Boehringer Ingelheim Ltd., Auckland, NZ). No further measurements were taken. The study was conducted over eight experimental repetitions, with one or two groups enrolled in each repetition.

2.2. Behavioural measurements

2.2.1. Behaviour

Behaviour was recorded continuously in real time (30 fps) by a security NVR system (ND9541, Vivotek, Taiwan) and 2 megapixel cameras (DS-2CD2432-F-IW, Hikvision, Hangzhou, China) fitted with 4 mm lenses. Cameras were attached to poles on the side of the pens and positioned 2 m off the ground. The cameras recorded in colour during the day and had built in near-infrared (NIR) lighting that allows for the capture of video in black and white during low-light/night-time conditions. Two cameras were positioned over each pen. All video footage was played back using VLC (Version 2.2.1, VideoLAN