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Clinical trial protocol: The effects of xylazine in 2-6 week old calves disbudded with cautery iron

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Administrative information

This trial protocol was written in accordance to Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 Checklist (Chan et al., 2012). Funding for this study was received from the Ontario Ministry of Agriculture, Food, and Rural Affairs – University of Guelph Research Program. Grant proposals were written by CBW in collaboration with TJD, KDL, and TFD. Study sponsors and funders were not/will not be involved in study design, data collection, data management, analysis and interpretation of data, writing of the report, and decision of submission and publication.

Introduction

Dehorning is well known to be a painful procedure commonly practiced in the dairy industry (Stock et al., 2013). Studies have found that the use of local anesthetic with NSAID analgesia can greatly decrease the amount of pain related outcomes observed in calves following cautery dehorning procedures such as pressure sensitivity, serum cortisol, and pain behaviour. (Duffield et al., 2010; Faulkner and Weary, 2000; Heinrich et al., 2010; Milligan et al., 2004). Longer-term pain management, such as an NSAID, has also been reported to benefit self-rewarding behaviours in calves such as play behaviours (Mintline et al., 2013). While the effects of local anesthesia and NSAID analgesia have been proven to be beneficial in reducing pain and stress for calves, the effects of sedation are not as well documented. A survey conducted among dairy producers and veterinarians in Ontario found that 62% of producers use a local anesthetic when dehorning, 24% report use of an NSAID while 38% report use of a sedative and 62% of veterinarians report use of a sedative (Winder et al., 2016). The use of sedation for dehorning is widely practiced, as well, it is recommended by the Canadian code of practice for the care and handling of dairy cattle to use sedation when disbudding or dehorning (NFACC, 2009), although there is not substantial evidence to base this off of. Studies on sedation reported that the most reliable biomarker of stress, serum cortisol, was higher in sedated calves (Stilwell et al., 2010) making it difficult to determine if a sedative is beneficial for reducing pain responses and stress. Therefore a well-designed trial assessing the effects of sedation for disbudding would be beneficial for the industry.

The objective of this study is to evaluate the effects of xylazine on outcomes associated with disbudding pain, behaviours, inflammation and stress responses in dairy calves 2-6 weeks

of age (feeding behaviour, milk consumption, pressure sensitivity, play behaviour, laying behaviour, serum haptoglobin, WBC differential and struggle behaviours).

Methods: Animals, interventions, assignment, and outcomes

This study will be conducted at the Elora Dairy Research and Innovation Centre with calves (both bulls and heifers) 2-6 weeks of age. Researchers will visit the centre to collect data every 3-4 weeks (when a room of calves is full at 15 calves) at -1, 0 and +1 days relative to the disbudding procedure. Calves will be randomly assigned to one of two treatments (60 calves per group, based on an expected difference in mean daily milk consumption between the treatment groups of 1.5L (8 vs. 6.5) with a SD of 3). Calves are eligible for trial if they have health scores (McGuirk) of equal to or less than: 3 (rectal temperature), 3 (ocular discharge, nasal discharge, cough score, and feces), 2 (ear position, naval, joint) and are not appreciably polled by palpation of the horn bud area. The research team will administer all interventions and will evaluate all outcomes.

Intervention groups

(1) XYLAZINE: lidocaine cornual nerve block (2% lidocaine HCl, 5 mL per side), meloxicam (0.5 mg/kg SQ), xylazine sedation (0.2 mg/kg IM), cautery iron

(2) NON-XYLAZINE: lidocaine cornual nerve block(2% lidocaine HCl, 5 mL per side), meloxicam (0.5 mg/kg SQ), cautery iron

Intervention allocation and blinding

Treatments will be randomly assigned and blocked by room/day; each day that trial is conducted, a full room of calves (maximum 15) will participate. Treatments will be assigned by use of a pre-made list with numbers 1-15 with the letters A and B in a different randomized order every day by use of a random number generator. Calf numbers will be written numerically from the youngest calf to the oldest on each list every trial day with calves assigned to the treatment (A or B) corresponding with their number on the list. Calves will be health scored and enrolled/not enrolled without knowledge of the treatment order sheet. The research team will be blinded to the xylazine treatment with the exception of a research technician who will know which treatment group receives xylazine and will administer the sedative while the other members are not present; this technician will not be involved in further treatment administration, evaluation of outcomes, or analysis.

Intervention administration

Xylazine IM injections and meloxicam SC injections will be administered 30 minutes prior to cautery procedure. Lidocaine blocks will be administered 20 minutes prior to cautery procedure. Hair on the horn buds will be clipped with clippers 24 hours prior to the procedure as well. Researchers will observe calves for a minimum of 4 hours post-disbudding.

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Outcomes

Study flow is describe in Figure 1.

1. *Feed and milk consumption* Automatic milk feeders and starter feeders will record data for each calf 48 hours prior to, and 48 hours post-disbudding. Data will be recorded on daily total intake, number of rewarded visits, number of unrewarded visits, average drinking speed, and average meal volume analyzed. Sample size was based on total amount consumed per day as the primary outcome.

2. *Pressure sensitivity* A pressure force algometer will be used as described by Winder et al. (2017), and readings will be taken at -24 hours, 0 minutes, +1 hour, +4 hours, and +24 hours relative to disbudding.

3. *WBC Differential* 10 mL of blood will be taken from the jugular vein via venipuncture at -24 hours, -2 hours, 0 minutes, +1 hour, +4 hours, and +24 hours relative to disbudding.

4. *Haptoglobin* 10 mL of blood will be taken from the jugular vein via venipuncture at -24 hours, -2 hours, 0 minutes, +1 hour, + 4 hours, and +24 hours relative to disbudding.

5. *Standing and laying behaviour* HOBO data loggers will be used as described by Winder et al. (2017) and will be attached to calves 24 hours prior to disbudding and removed 24 hours following disbudding. Data for each day will be analyzed separately, with total time spent laying, number of laying bouts, and average laying bout length offered for analysis.

6. *Play behaviours* Play behaviours will be induced with the use of new bedding (wood shavings) and will be video recorded for 15 minutes at -24 hours, +3 hours, and +24 hours relative to disbudding. Play behaviours that will be recorded will be running, bucking and head to head contact as described in Winder et al. (2017).

7. *Escape-related behaviours during procedure* During the disbudding procedure calves will be video recorded individually to assess and record escape-related behaviours during disbudding. Behaviours that will be recorded will include vocalizing, kicking, struggling, falling, and leg movements while lying down (Table 1).

8. *Time to administer nerve blocks* During the administration of the lidocaine cornual nerve blocks, a stopwatch will be used to determine the length of time it takes to block each calf from the insertion of the needle on the right side to the removal of the needle on the left side.

9. *Time to disbud* Each calf will be timed during the disbudding procedure to determine the length of time it takes to remove both horn buds beginning with the

placement of the iron on the right side of the head to the removal of the iron on the left side of the head. During this time all blinded researchers will guess whether they think the calf was sedated or not.

Methods: Data collection, management, and analysis

CBW will train the research team, and one assessor will take all algometry readings and perform every disbudding procedure. Baseline values will be taken for feed and milk consumption (-48 hours), play behaviours (-24 hours), pressure sensitivity (-24 hours), health scoring (-24 hours), haptoglobin (-24 hours, -2 hours), WBC differential (-24 hours, -2 hours), and standing and laying behaviour (-24 hours). Algometry readings, health scores, time to block, sedation estimates and any additional notes for each day will be recorded on paper by the researchers and will be stored and kept in a locked lab at the end of each collection day. Data will then be transferred to an excel file and checked for errors prior to cleaning. At +24 hours relative to disbudding, HOBO loggers will be removed; this data will be kept on a research computer as well as on a USB stick in a locked lab until it can be analyzed. Blood samples will be kept on ice during the trial day and collection days. Serum samples for haptoglobin will be spun down by 6 hours post-collection, kept at -20C and run as a single batch using the Roche Cobas 6000 c501 and biochemistry analyzer with methemoglobin stock reagent using formulas and operating conditions developed by J.G. Skinner laboratory, Veterinary Investigation Centre (Aberdeen, Scotland). Samples for WBC differential determination will be run on the Qscout BLD and blood smears will be made for each sample at the end of each trial day.

Statistical analysis

Data will be exported into STATA15 (Stata/IC Version 15.1 for Mac, StatCorp, College Station, TX) and descriptive statistics reviewed for normality and variation. Univariate association of treatment group on outcome will be assessed for binary outcomes with logistic regression, continuous outcomes with linear regression, and count data with both Poisson and negative binomial models, with the negative binomial model used if the over-dispersion term is significant. Zero-inflated models will be built if there are a large number of zeros in the data. Baseline data will be used as a fixed effect and replicate number as a random effect. For outcomes with repeated measures, calf will be included as a random effect, time as a fixed effect, and a treatment by time interaction will be examined.

Ethics and dissemination

Ethical approval has been obtained prior to the start of trial (AUP#4060). Changes to the protocol will be reported as protocol deviations in the relevant manuscript. All calves will be receiving industry standard for best practice when disbudding, as they will all receive both cornual nerve blocks and NSAID treatment. Results will be analyzed during the fall of 2019 and should be submitted for publication by spring of 2020.

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Table 1. Escape behaviour during disbudding

Behaviour	Description
Vocalizing	Any audible noise including bawling and moaning
Kicking	Sharp backwards or sideways extension of the hind right or left leg
Struggling	Any sharp or abrupt movements or behaviours including lunging forward, backward or sideways while standing and rapid head movements in any direction
Falling	A rapid or abrupt downward movement of the body involving the animal collapsing where one or both of its hips meet the ground first
Laying down	A controlled downward movement involving the animal dropping onto the front knees first and hips second
Kicking while laying down	Rapid outward extensions of one or more of the right or left hind or front legs while the animal is laying down

Figure 1. Study flow