ABSTRACT

Cauterization by hot iron and application of caustic paste are 2 common methods of disbudding calves. In this study, we compared the affective experience of these 2 procedures on young dairy calves using conditioned place aversion. Male dairy calves (n = 14; 7 ± 2 d old) were disbudded by both thermal and chemical methods (1 horn bud at a time, 48 h apart). Calves received treatments in pens made visually distinct with either red squares or blue triangles on the walls. Calves were restricted to these treatment pens for 6 h following disbudding. For all treatments, calves received a sedative (xylazine, 0.2 mg/kg), local anesthetic (lidocaine, 5 mL), and analgesic (meloxicam, 0.5 mg/kg). Calves were then tested for conditioned place aversion at 48, 72, and 96 h after their last treatment. During tests, calves were placed in a neutral pen connected to both treatment pens where they had previously been disbudded. Time spent in each treatment pen was recorded until calves chose to lie down for 1 min (latency to lie down: 31.0 ± 8.6 min). During the first test (48 h after last disbudding), calves spent more time in the pen associated with hot-iron disbudding compared with what would be expected by chance (intercept: 73.5%, 95% CI: 56.5, 90.5) and fewer calves lay down in the caustic paste pen than in the hot-iron pen (3 vs. 10 lying events). No evidence of preference for the hot-iron pen was found in the following test sessions (72 and 96 h since last treatment). These results suggest that calves initially remember caustic paste disbudding as a more negative experience than hot-iron disbudding, even with the use of sedation, local anesthesia, and analgesia.

Key words: cautery, dehorning, pain, affect, nonsteroidal anti-inflammatory drug (NSAID)

INTRODUCTION

Disbudding is common on dairy farms (Cozzi et al., 2015; USDA, 2018). The use of an iron rod heated to a high temperature (500–600°C) to cauterize horn tissue is the most common procedure, with around 70 to 80% of North American and European farms using this method (Cozzi et al., 2015; Winder et al., 2016; USDA, 2018). Hot-iron disbudding (“dehorning” when performed on older calves with developed horns) is a painful procedure, but the pain can be mitigated with local anesthetics and postprocedural pain control (for reviews, see Stafford and Mellor, 2011; Herskin and Nielsen, 2018; Winder et al., 2018).

An alternative method of disbudding is the application of a caustic paste (often a mix of calcium hydroxide and sodium hydroxide) that results in a chemical burn that damages the developing tissue. Caustic paste is less commonly used than hot-iron disbudding; approximately 10 to 30% of farms in North America and Europe use this method (Gottardo et al., 2011; Cozzi et al., 2015; Winder et al., 2016; Staněk et al., 2018; USDA, 2018); however, the proportion of heifers in the United States disbudded with caustic paste increased from 12 to 32% between 2007 and 2014 (USDA, 2009, 2018).

Studies have reported pain-related behaviors (such as ear flicks and head shakes), increased cortisol, and increased pressure sensitivity in the hours following caustic paste application, and that local anesthesia and a nonsteroidal anti-inflammatory drugs (NSAID) can mitigate these responses (Stilwell et al., 2009; Winder et al., 2017). Two studies directly compared caustic paste to hot-iron disbudding, but the results of these comparisons were inconsistent. Morisse et al. (1995) found that chemically disbudded calves showed a higher cortisol response than cauterized calves. In contrast, Vickers et al. (2005) found more pain-related behaviors following hot-iron disbudding than following caustic paste disbudding.

In previous applications of conditioned place aversion, calves spent less time and were less likely to lie down in a pen associated with cautery disbudding com-
pared with a pen where they were only sedated (Ede et al., 2019c). Calves also showed less aversion to cautery disbudding if they received the NSAID meloxicam (compared with not receiving it) in addition to sedation and local anesthesia (Ede et al., 2019a).

The objective of this study was to use conditioned place aversion to compare the experience of caustic paste to hot-iron disbudding, in both cases with sedation, local anesthesia, and an NSAID.

**MATERIALS AND METHODS**

This study was approved by University of British Columbia’s Animal Care Committee (Protocol A16–0310) and was conducted at University of British Columbia’s Dairy Education and Research Centre in Agassiz, Canada from October to December 2019.

**Animals and Housing**

Fifteen Holstein bull calves were enrolled at (mean ± SD) 7 ± 2 d of age, weighing 51 ± 4 kg. Calves were individually housed in 2.1- × 2.4-m pens bedded with sawdust, fed 4 L of whole milk twice a day (at approximately 0900 and 1600 h), and given ad libitum access to water, hay, and grain.

**Apparatus**

The apparatus was similar to the one described in previous studies (Ede et al., 2019a,c). Briefly, a 2.1- × 6.0-m pen with plywood walls was divided in 3 pens (2 “treatment” pens and a “central” pen, 2.1- × 2.0-m each), bedded with sawdust and separated by removable gates (Figure 1). As visual cues to aid place association, colored sheets (either blue triangles or red squares) were fixed on the walls of the treatment pens. A holding chute mounted with a bottle holder was positioned in front of the entry gate leading into the central pen.

**Pre-exposure**

Calves were individually pre-exposed to the apparatus before enrollment. During pre-exposure, calves were brought from their home pen (at approximately 1100 h) to the holding pen, placed in front of the apparatus, and given a milk reward (approximately 0.3 L of whole milk). Calves were then let into the apparatus (gates had been removed, allowing access to all pens). Time spent in each treatment pen (i.e., with both front legs in the pen) was recorded for 15 min, after which calves were brought back to their home pen. To avoid including animals with a strong pre-existing bias, one calf that did not enter both treatment pens during pre-exposure was excluded, resulting in a total of 14 subjects.

**Treatments**

At 24 and 72 h after pre-exposure (to allow a 2-d recovery from disbudding), calves were disbudded (one horn bud at a time, one in each treatment pen, such that all calves received both treatments). During hot-iron disbudding, calves were brought to the holding chute where they received a milk reward (approximately 0.3 L) as well as a subcutaneous injection of sedative (xylazine 0.2 mg/kg, Rompun 20 mg/mL, Bayer, Leverkusen, Germany), which was expected to sedate calves for approximately 1 to 2 h (Ede et al., 2019b). We did not expect xylazine to prevent calves from learning to associate place with treatment, as calves have previously shown evidence of conditioned place aversion following xylazine treatments (Ede et al., 2019a,c). Calves were then led to one of the treatment pens (with the gate mounted, confining the calf to that pen). Once the calves were sedated (recumbency and eyeball rotation, 5–10 min after injection), they were injected with a local cornual nerve block (5 mL, lidocaine 2%, epinephrine 1:100,000, Lido-2, Rafter8, Calgary, AB, Canada) in the lateral canthus of the eye of the side to be disbudded. Immediately after the local block, calves were injected with an NSAID subcutaneously in the neck (meloxicam 0.5 mg/kg, Metacam 20 mg/mL, Boehringer Ingelheim, Burlington, ON, Canada). At 10 min after injection of the local block, the horn bud was shaved, tested for pain reflex with a
needle-prick (no calf reacted), and cauterized with a preheated hot iron (X30, 1.3 cm tip, Rhinehart, Spencer-cville, IN) for approximately 10 s (no calf reacted to disbudding). Calves were then positioned in sternal recumbency and left in the treatment pen for 6 h, a duration long enough to allow calves to recover from sedation and local anesthesia, which are both expected to be effective for approximately 1 to 2 h (Winder et al., 2018; Ede et al., 2019b). Calves were then brought back to their home pen.

For caustic paste disbudding, all aspects of the procedure were identical, but instead of using a hot iron, a thin layer of disbudding paste (calcium hydroxide 24.9%, sodium hydroxide 21.5%, Dr. Naylor, Morris, NY) was applied onto the horn bud (as a circle of around 2 cm in diameter and approximately 1 mm thick) and a ring of petroleum jelly (Original Vaseline, Unilever, Toronto, ON, Canada) was applied around the horn bud.

Order of treatment, color of treatment pen, side of horn bud, and pre-exposure preferences were counter-balanced among calves by Latin square design.

Tests

At 48, 72, and 96 h after the second disbudding treatment, calves were tested for place aversion. During tests, gates were removed, allowing calves access to all pens. Time spent in each treatment pen (i.e., with both front legs in that pen) was recorded live from video (camera: WV-CP310, Panasonic Canada, Mississauga, ON, Canada) until the calf chose to lie down for at least 1 min, ending the session. All calves lay down within 60 min of the test start.

Statistical Analysis

A minimum sample size of 10 animals was calculated using the “power.t.test” function in R (https://www.R-project.org/) for a statistical power of 0.8 and a significance level of 0.5, given a mean difference in time spent between treatment pens equal to the standard deviation of the difference.

Before Conditioning. The proportion of time spent in the pen to be associated with hot-iron disbudding (of total time spent in both treatment pens) during pre-exposure was compared with the null expectation (50%) using a one-sample t-test; normality of the differences was confirmed graphically.

After Conditioning. The proportion of time spent in the hot-iron pen was analyzed with a linear mixed model (Bates et al., 2015), testing the fixed effects of test session number (1, 2, or 3; 1 df) order of treatment (hot iron or caustic paste first, 1 df), their interaction (1 df), color of the pen associated with hot-iron disbudding (blue triangles or red squares, 1 df), horn side disbudded by cautery (left or right, 1 df), and latency to lie down (continuous, 1 df) with calf specified as a random effect. Normality and homoscedasticity of residuals were confirmed graphically. Reported estimates for fixed factors reflect the difference in proportion of time spent in the hot-iron pen between factor levels; 95% confidence intervals were obtained with the “confint” function in R. An effect of test session was found, but no other factor was statistically significant (see Results section). An additional model that did not include nonsignificant factors (P > 0.1) was also conducted (fixed effect: test session number, random effect: calf). Fit of the full and simplified models was compared by ANOVA, and results from both models are reported.

The effect of treatment on the pen in which calves chose to lie down was analyzed using the chi-square test, separately for each of the 3 postconditioning test sessions. No adjustment for multiple comparisons was made due to our small sample size. We encourage future research to take this into consideration in power calculations. Data and R code are freely accessible in Supplemental Table S1, File S1, and File S2 (https://doi.org/10.3168/jds.2020-18299).

RESULTS

Before conditioning, the proportion of time spent in the hot-iron pen did not differ from the 50% value expected by chance (47.3%, 95% CI: 32.9, 61.7; P = 0.7).

After conditioning, calves took an average of 31.0 (±8.6) min to lie down. Proportion of time spent in the hot-iron pen did not vary in relation to treatment order or its interaction with test number (hot iron first: −2.4%, 95% CI: −35.7, 30.7; P = 0.9; interaction with test number: +5.0%, 95% CI: −9.4, 19.6; P = 0.5), color of the pen associated with hot-iron (red squares: −10.3%, 95% CI: −28.5, 7.9; P = 0.3), side of horn bud treated with hot iron (right horn: −2.3%, 95% CI: −20.8, 16.2; P = 0.8) or latency to lie down (per minute: +0.01%, 95% CI: −0.8, 0.9; P > 0.9). However, the proportion of time spent in the hot-iron pen did vary in relation to test session; preference for this pen declined over the 3 test sessions (per session: −13.3%, 95% CI: −23.5, −3.2; P = 0.02; Figure 2a).

A simplified model only including test session number as fixed effect and calf as random effect was conducted. Excluding nonsignificant covariates did not hinder the model’s fitness (ANOVA: Χ2 = 3.0, P = 0.7). Calves initially spent more time in the hot-iron pen (intercept: 73.5%, 95% CI: 56.5, 90.5; P < 0.01), and preference for this side declined over the 3 sessions (per session: −10.8%, 95% CI: −18.1, −3.6; P < 0.01).
Fewer calves lay down in the pen associated with caustic paste disbudding during the first test session (23% of calves, $\chi^2 = 3.8, P = 0.05$), but we saw no evidence of preference in the second or third session (respectively, 33% of calves, $\chi^2 = 1.3, P = 0.2$; 50% of calves, $\chi^2 = 0, P = 1$; Figure 2b).

**DISCUSSION**

After disbudding with hot iron and caustic paste in distinct pens, calves spent less time and lay down less frequently in the pen associated with caustic paste compared with hot iron during the initial place aversion testing (48 h after last disbudding). These results suggest that, despite using the identical pain control protocol thought to be effective in mitigating the pain associated with both methods of disbudding (Stilwell et al., 2009; Winder et al., 2017), the caustic pain procedure was initially more aversive to calves.

We found no evidence of aversion in the following test sessions (72 and 96 h since last disbudding); reduced place aversion over time is expected when animals are tested repeatedly, as the learned association between treatment and pen is weakened every time the animal experiences the pen without painful stimulus (Brush, 1971). Reduced evidence of place aversion with multiple tests was also reported in a previous study investigating calf aversion to disbudding (Ede et al., 2019c). Motivation to spend more time in a pen they had previously avoided could also potentially be an expression of curiosity (Byrne, 2013) but this interpretation is speculative and we suggest that further work is required to determine whether this result is replicable.

Comparisons across studies are hindered by differences in calf age and pharmacological treatments. Also, the results of previous work are inconsistent. Vickers et al. (2005) compared calves disbudded by hot iron (treated with a sedative and local anesthesia) with those disbudded by caustic paste (treated with a sedative only) and found that calves in the caustic paste treatment showed less evidence of pain (as evidenced by fewer head shakes) in the first 4 h after the procedure. In contrast, Morisse et al. (1995) reported a weaker reaction to hot-iron disbudding than to caustic paste, although treatment was confounded by calf age. A series of studies on goat kids reported evidence of higher pain sensitivity (Hempstead et al., 2018c) and behavioral (Hempstead et al., 2018a) and physiological (Hempstead et al., 2018b) responses to chemical versus hot-iron disbudding, but no pain control was provided in any treatment. These results, combined with the mixed results from the current study, make it difficult to draw firm conclusions. However, the consistent results of the studies by Hempstead et al. (2018a,b,c), combined with aversion in the pen calves chose to lie down in during the initial test session of the current study, provides a tentative basis for recommending the hot-iron procedure over caustic paste. Even with a sedative, local anesthetic, and an NSAID, both methods of disbudding were likely aversive to calves, underlying the importance of developing further refinements for disbudding and, where feasible, avoiding this practice (e.g., by using polled sires).

Our study had several limitations. First, despite selecting our sample size based on a power analysis, our ability to detect differences, if they truly existed, was still limited by the number of calves tested. A second limitation was that aversion tests were based upon the calves’ memory of the 6 h following the procedure, which is unlikely to encompass the full duration of pain caused by disbudding (Winder et al., 2018; Casoni et al., 2009).
et al., 2019). The long duration of postoperative pain means that calves may still have been in pain from the first event during the second disbudding event. That said, we saw no evidence that this ongoing pain influenced aversion, as this would have been expected to result in an effect of treatment order. Third, we did not explore state-dependent effects (i.e., that the calves’ current pain state influences their aversion; see Tzschentke, 1998). Such effects have not been investigated in calves, but it is likely that calves avoided the pen associated with caustic paste not only because of their aversive memory of the procedure, but also because they were still feeling pain during testing. Fourth, our conclusions are limited to the overall aversiveness of the experience, and we cannot make inferences about more limited time frames (e.g., whether caustic paste was more aversive throughout conditioning or during only the last hours). Finally, our results are limited to the specific combination of pain control methods provided; the drugs used in this study are based upon a substantial body of research on hot-iron disbudding, but much less is known about pain control for caustic paste; it is likely that the ideal multi-modal approach will differ for these 2 procedures.

CONCLUSIONS

Calves tended to spend less time and lay down less often in a pen associated with caustic paste disbudding compared with hot-iron disbudding, but only in the first test session (48 h after last disbudding); results from following test sessions (72 and 96 h after last disbudding) were ambiguous. Our results suggest that caustic paste is initially more aversive than hot-iron disbudding, but this finding should be considered tentative and warrants further investigation.

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