ABSTRACT

We evaluated alternatives to cautery disbudding of goat kids using physiological measures of immediate and longer-term pain. Fifty Saanen doe kids were randomly assigned to 1 of 5 treatments (n = 10/treatment): (1) cautery disbudding (CAUT), (2) caustic paste disbudding (CASP), (3) liquid nitrogen disbudding (CRYO), (4) clove oil injected into the horn bud (CLOV), or (5) sham disbudding (SHAM). Serum cortisol and haptoglobin concentrations were measured from blood samples collected immediately before treatment (baseline) and at 15, 30, 60, and 120 min and then again at 6 and 24 h post-treatment. An infrared thermography camera was used to take images of the horn buds 24 h pre- and 24, 48, and 72 h post-treatment to measure skin temperature. Body weight was measured daily for 1 wk to assess weight change post-treatment. Images of the horn buds were taken at 1, 2, and 7 and at 6 wk post-treatment to assess tissue damage and wound healing. Mean cortisol concentrations were elevated in CASP kids 1 h post-treatment relative to CAUT kids. Cortisol concentrations of CRYO kids were higher than those of CAUT kids 30 min post-treatment; concentrations for CLOV kids were similar to CAUT kids post-treatment. Mean haptoglobin concentrations were similar across treatments over time; however, CLOV kids had higher concentrations at 24 h post-treatment than all other treatments. Skin temperatures of CASP and CLOV kids were elevated relative to CAUT kids at all time points post-treatment, and all disbudded kids had skin temperatures above those of SHAM kids at 72 h post-treatment. Treatment did not influence weight gain. The CAUT kids had large, open wounds exposing bone; small scabs were still evident 6 wk post-treatment. The CASP kids had red and open, raw wounds that generated large eschars, apparent for up to 6 wk. The CRYO kids had closed, dry wounds initially, but over time lesions appeared that caused open wounds; small scabs were present 6 wk post-treatment. The CLOV kids had closed, dry wounds with blackened skin; healed skin and minimal scabs were present 6 wk post-treatment. Caustic paste and cryosurgical disbudding appeared to cause more pain compared with cautery disbudding; thus, these methods may not provide good alternatives to cautery disbudding. Clove oil appeared to cause a similar pain response as cautery disbudding and smaller wounds with earlier tissue repair; this method shows promise as an alternative to cautery disbudding. Key words: caustic paste, liquid nitrogen, clove oil, cortisol

INTRODUCTION

Disbudding of goat kids and calves is performed to prevent horn growth, as horns can cause injuries to other farmed animals and human handlers. Cautery disbudding of kids is a common practice but is painful and can cause health issues (Thompson et al., 2005; Alvarez et al., 2009; Hempstead et al., 2017). Pain in disbudded kids can be assessed using behavioral and physiological responses such as frequent intense vocalizations (Alvarez and Gutierrez, 2010), increased frequencies of head and body shaking, longer bouts of head scratching (Greenwood and Shutt, 1990; Hempstead et al., 2017, 2018), and elevated plasma β-endorphin (Greenwood and Shutt, 1990) and cortisol concentrations (Alvarez et al., 2009, 2015; Hempstead et al., 2018). Calves show defensive behavior during disbudding, such as rearing or dropping to the ground, and responses post-procedure, such as head shaking and ear flicking (Graf and Senn, 1999; Grondahl-Nielsen et al., 1999; Heinrich et al., 2010). Moreover, disbudded calves display elevated cortisol concentrations and heart and respiratory rates (Grondahl-Nielsen et al., 1999; Heinrich et al., 2009), a more variable heart rate, a rapid decrease in eye temperature (Stewart et al., 2008), reduced weight gain when pain relief is unavailable (Bates et al., 2016), and
heightened sensitivity to pressure applied to the disbudding wounds (Heinrich et al., 2010).

Haptoglobin is an acute phase protein that is produced in response to inflammation and has previously been measured in disbudded calves provided nonsteroidal anti-inflammatory drugs (NSAID; Allen et al., 2013). Infrared thermography has also been used in cattle to measure inflammation; higher skin temperatures in tissues of either hot-iron or freeze-branded cattle were found relative to unbranded controls measured at the same position (Schwartzkopf-Genswein and Stookey, 1997). Currently, research assessing inflammatory responses to disbudding in kids is limited. Hence, there is a need to evaluate pain mitigation strategies and alternative methods to cautery disbudding that can cause less pain and less tissue damage and improve wound healing.

Alternative methods to cautery disbudding have been evaluated in calves but have not been assessed in kids. Methods include the application of caustic paste (Morisse et al., 1995; Vickers et al., 2005; Stilwell et al., 2009), liquid nitrogen (i.e., cryosurgical disbudding; Bengtsson et al., 1996; Stewart et al., 2014), and clove oil (Molaei et al., 2014) to horn buds. Caustic paste techniques involve the application of a sodium, calcium, or potassium hydroxide paste that chemically burns the horn bud area; application of these pastes has been reported to be less painful than cautery disbudding in calves based on lower frequencies of head shakes (Vickers et al., 2005). Furthermore, the frequencies of pain-related behaviors (e.g., head rubs, head shakes, tail flicks) were much lower in calves disbudded with caustic paste and a local anesthetic cornual block than in calves treated with caustic paste alone (Winder et al., 2017). Cryosurgical disbudding involves spraying liquid nitrogen on the horn buds to destroy cells and may be less painful than cautery disbudding as it causes a reduced inflammatory response (Bengtsson et al., 1996; Stewart et al., 2014). Clove oil has traditionally been used in dentistry as a mild topical anesthetic (Chaieb et al., 2007). Furthermore, clove oil is a well-established fish anesthetic (Sladky et al., 2001). Recent studies on calves (Molaei et al., 2014) and goat kids (Molaei et al., 2015) have used clove oil as a novel method of disbudding and have shown arrested growth in horn buds injected with clove oil compared with saline injection. Clove oil, which contains eugenol, has properties (e.g., cytotoxic, necrotizing agent, anesthetic; Markowitz et al., 1992; Prashar et al., 2006) that may have application for disbudding. These methods have the potential to improve goat kid welfare compared with cautery disbudding.

We evaluated alternatives to cautery disbudding (i.e., the application of caustic paste, liquid nitrogen, and clove oil) for goat kids using physiological measures of immediate and longer-term pain. We predicted that the application of caustic paste would cause the greatest pain response and that cryosurgical disbudding and clove oil injection would have the lowest pain response relative to cautery disbudding. We also expected caustic paste to cause the greatest tissue damage and that kids injected with clove oil would benefit from the anesthetic properties of eugenol, a main component of clove oil (Markowitz et al., 1992).

MATERIALS AND METHODS

Animals and Housing

Our study was conducted on 50 female Saanen and Saanen cross dairy goat kids (mean ± SD = 5.2 ± 0.66 kg) aged 9 to 14 d (mean ± SD = 10.6 ± 0.91 d) at the Ruakura Research Farm, Waikato region (37°47′S, 175°19′E), New Zealand, during July and August 2016. The study was approved by the Ruakura Animal Ethics Committee (protocol no. 13899). All kids received goat colostrum at birth and were separated from their dam after 24 h. Kids were transported to the research farm when approximately 2 d old. Once kids arrived, they were weighed and given an identification collar. Kids were also vaccinated subcutaneously (Covexin, Schering-Plough Animal Health Ltd., Wellington, New Zealand) per routine farm practice and prophylactically administered an antibiotic subcutaneously (Norocillin, 30% wt/vol, Norbrook Laboratories Ltd., Northamptonshire, UK).

The animals were housed in groups of 5 in pre-treatment pens (2.4 × 1.6 m). The pen floors were covered with clean, dry bedding (wood shavings, PGG Wrightsons, Hamilton, New Zealand) 10 cm deep. The kids remained with the same pen-mates for the entire trial. Kids were habituated to the pens for 1 d before baseline (pre-treatment) data collection. Kids had access to at least 600 mL of milk replacer/d per kid, which was increased over the study period to 1 L/d per kid (Anlamb, Fonterra Ltd., Auckland, New Zealand), via a feeder. Fresh water was supplied in a bucket attached to the pen wall. Overnight, feeders were removed to reduce gut fill affecting BW measurements the following morning. The feeders were then replaced at approximately 0700 h. Once the BW measurements concluded at 2 wk post-treatment, feeders were left in the pens overnight. Daily temperature and relative humidity within the goat barn ranged between 6.0 and 24.5°C (12.9 ± 0.03°C) and 93 and 37% (69.5 ± 0.18%), respectively.
**Experimental Design**

We used a randomized complete block design blocked by treatment day and pen within treatment day. Kids were randomly allocated to 1 of 5 treatment groups balanced for age (n = 10/treatment). Only 1 kid per treatment was represented in each pen. The experimental component of the study was conducted over 4 treatment days within a 2-wk period. Each kid was fed approximately 1 h before treatment and then collected from their pre-treatment pen and placed into a restraint device, which held the animal 90 cm off the ground for operator ease. The device consisted of a rigid plastic pipe (0.35 m × 0.85 m) sectioned lengthways with holes for the kids' legs and 2 straps (Velcro, London, UK) used to secure the kid across the shoulder and back. Treatments were carried out in the same room containing the pre- and post-treatment pens. The order of treatment was randomly assigned. Hair covering the horn buds was removed with an electric clipper (Laube, 505 cordless kit, Shoof International, Cambridge, New Zealand) to expose the horn buds. Treatments were performed by the same veterinarian between 0900 and 1030 h each day and included the following:

1. Sham treatment (SHAM): Kids were sham-handled and a finger was used to massage each horn bud in a circular motion for 10 s (i.e., not disbudded).

2. Cautery disbudding (CAUT): Kids were cauter-disbudded using an iron (Quality electric debudder, 18-mm tip, 230 V, 190 W; Lister GmbH, Lüdenscheid, Germany), which was heated to approximately 600°C for 20 min before being held to each horn bud for 5 to 7 s. Horn buds were then removed by pressing the iron down and then rotating it so the skin was cut and the buds forcibly flicked out. The cautery disbudding treatment was modified from the procedure described by Hempstead et al. (2017).

3. Caustic paste (CASP): Kids were disbudded using a sodium hydroxide-based caustic paste (Hornex, Shoof International Ltd.) that was rubbed onto each horn bud (0.16 mL/bud) using a fingertip on a gloved hand. A ring of petroleum jelly was spread around each horn bud before application to stop the paste from running into the kids’ eyes. The caustic paste treatment was modified from the protocol described by Vickers et al. (2005) for calves.

4. Liquid nitrogen (CRYO): Kids were cryosurgically disbudded using a commercial applicator (CryAc B-700, 500-mL capacity, Brymill Cryo-

No pain mitigation was provided as part of any treatment because the aim of the study was to evaluate the pain response relative to the different procedures. After treatment, the CAUT kids’ wounds were sprayed with antibacterial spray (AluSpray, Neogen Corp., Lexington, KY) to prevent infection. The horn buds of CASP, CRYO, and CLOV kids were not sprayed to avoid interference with the treatments. The animals were then placed into their post-treatment pens (adjacent

**Figure 1.** Needle position used to inject clove oil into kids’ horn buds. Illustration created by Chelsea Dela Rue. Color version available online.
to their pre-treatment pens). The kids remained at the research farm in holding pens until they were returned to the farm of origin 6 wk after treatment, once health monitoring had concluded.

**Blood Sampling**

Serum cortisol and haptoglobin concentrations were measured from 4-mL blood samples collected by venipuncture from both jugular veins immediately before treatment; at 15, 30, 60, and 120 min; and at 6 and 24 h following treatment. Each kid was firmly restrained by a handler while samples were collected using 22-G, 2.54-cm needles (PrecisionGlide, Becton Dickinson, Franklin Lakes, NJ) and kept in serum tubes (Becton Dickinson). Blood samples were centrifuged at approximately 1,500 × g for 10 min at 20°C after leaving the samples to sit for approximately 1.5 h. The serum was separated and stored at −20°C until analyzed.

Samples were analyzed by a commercial laboratory using standard quality control methodologies; technicians were blind to the treatment each kid received. Cortisol concentrations were determined by electrochemiluminescence immunoassay using a commercial kit (Roche Diagnostics GmbH, Mannheim, Germany). Sensitivity of the assay was 1.5 nmol/L. Haptoglobin concentrations were determined by colorimetric assay using a Tridelta phase haptoglobin kit (Tridelta Development Ltd., Maynooth, Ireland). Sensitivity of the assay was 0.005 mg/mL.

**Skin Surface Temperature**

Images of the horn buds were taken using a handheld infrared thermography camera (FLIR T650sc; FLIR Systems Inc., Wilsonville, OR). Two images were taken immediately before treatment (0900 h) and again at 24, 36, and 72 h post-treatment (~1500 h), and the clearest image of the pair at each sampling point was selected for analysis. Images were not taken within 24 h of treatment to avoid handling and associated stress affecting other measures. Kids were restrained so that the head remained horizontal to the floor, and images of the horn bud area were taken (~3-cm diameter around each horn bud). Handlers took care not to touch the horn bud area during restraint. All images were taken at 1 m directly in front of the animal. Ambient temperature (°C) and relative humidity (%) within the facility were recorded continuously using a data logger (EL-USB-2-LCD+, Lascar Electronics, Salisbury, UK), and temperature and humidity data were entered into the ThermaCam Researcher Pro 2.10 software (FLIR Systems AB, Täby, Sweden) used for thermal image analysis. The maximum skin surface temperature (°C) was calculated from an approximately 2-cm-diameter area surrounding each horn bud.

**BW Measurements**

Kids were weighed daily before feeding; this was done for 3 d before treatment, on the day of treatment, and daily for 1 wk after treatment. A final weight measurement was taken 2 wk after treatment. Kids were placed in a large fabric bag and weighed using free-standing digital hanging scales (model WS603, Wedderburn, Auckland, New Zealand).

**Healing Images**

Images of the horn bud area of each goat kid were taken using a Nikon camera (Coolpix L840; Nikon Corp., Tokyo, Japan) at d 1, 2, and 7 and at 6 wk after treatment to provide visual aids for descriptions of wound healing over time. The following criteria were used to assess tissue damage and wound healing: (1) whether the wound was open (broken skin with dermal layers visible) or closed (skin intact), (2) the presence of fluid (wet or dry), (3) whether an eschar (brown or black deep tissue damage that is flush with skin) or a scab (brown, thin layer of dried blood cells that sits on top of the skin surface) was present, and (4) the color of tissues (i.e., redness/erythema or blackened).

**Statistical Analysis**

Data were analyzed using GenStat software (version 17, VSN International, Hemel Hempstead, UK). Residual plots were assessed to detect departures from the assumptions of normality and constant variance. No transformations were required. One animal from the CAUT group was removed from the trial due to a leg injury not related to treatment. Serum cortisol and haptoglobin concentrations and BW were analyzed using a repeated measures model fitted by REML. The model included fixed effects for treatment, time (day for BW), and their interaction and random effects for treatment day, pen, kid, and age. The correlation in measurements taken on the same kid over time was modeled with a power model of order 1 for plasma cortisol and haptoglobin concentrations and for BW. The model for skin surface temperature included the same fixed and random effects as above with the addition of random effects for horn bud (left or right) within kid. The correlation in measurements taken on the same horn bud over time was modeled with an autoregressive model of order 1. Differences between and within treat-
ments were detected using Fisher’s least significant differences test. Mean values were provided with standard errors of the difference. The level of significance was set at $P \leq 0.05$.

RESULTS

Blood Sampling

There was a treatment by time interaction for cortisol concentrations ($F_{24,163} = 6.6, P < 0.001$; Figure 2). Mean baseline cortisol concentrations were not different across treatments [27.8, 31.4, 27.7, 25.4, and 28.3 ± 10.89 nmol/L (pooled SE of the difference) for SHAM, CAUT, CASP, CRYO, and CLOV kids, respectively; $P > 0.50$]. Mean cortisol concentrations did not differ across time for SHAM kids ($P > 0.10$) except at 2 h, where cortisol concentrations were elevated above baseline levels ($P \leq 0.05$). Mean cortisol concentrations of CAUT kids increased from baseline ($P > 0.01$) and were not different from SHAM kids 15 min post-treatment ($P > 0.10$). Mean cortisol concentrations of CLOV kids increased from baseline 15 min post-treatment ($P \leq 0.01$) and did not differ from those of SHAM kids for up to 1 h post-treatment ($P > 0.10$). Furthermore, CLOV kid cortisol concentrations were not different from those of CAUT kids over 24 h post-treatment ($P > 0.10$). Mean cortisol concentrations of CRYO kids were elevated above baseline ($P \leq 0.01$) and CAUT kid levels 30 min post-treatment ($P \leq 0.05$). Mean cortisol concentrations of CASP kids were elevated above baseline and CAUT kid levels for up to 1 h post-treatment ($P \leq 0.01$). By 6 h post-treatment, there were no differences between groups for mean cortisol concentrations, which had returned to baseline levels ($P > 0.10$).

A treatment by time interaction was observed for haptoglobin concentrations ($F_{24,181} = 10.9, P < 0.001$; Figure 3). Mean baseline haptoglobin concentrations were similar across treatments [0.43, 0.53, 0.50, 0.37, and 0.37 ± 0.095 g/L (pooled SE of the difference) for SHAM, CAUT, CASP, CRYO, and CLOV kids, respectively; $P > 0.05$]. For SHAM kids, haptoglobin concentrations did not differ over time ($P > 0.50$). Post-treatment haptoglobin concentrations for CAUT kids were not different from baseline concentrations ($P > 0.10$). Kids experiencing CRYO and CASP treatments had higher haptoglobin concentrations than kids experiencing the other treatments at 24 h post-treatment ($P \leq 0.01$).
had no difference in mean haptoglobin concentrations across time ($P > 0.10$).

**Skin Temperature**

Due to missing baseline data, the skin temperatures from 21 kids were excluded from analysis. There was a treatment by time interaction for change in maximum skin temperature ($F_{8, 75} = 2.8; P = 0.009$; Figure 4). Skin temperature of CASP kids was warmer than SHAM kids for 72 h post-treatment ($P \leq 0.05$). The change in skin temperature did not differ between CAUT and SHAM kids up to 48 h post-treatment ($P > 0.50$); however, at 72 h post-treatment, the increase in skin temperature was higher in CAUT than SHAM kids ($P \leq 0.05$). The change in skin temperature did not differ between CRYO and SHAM kids up to 48 h post-treatment ($P > 0.10$); however, at 72 h post-treatment, the increase in skin temperature was higher in CRYO kids than in SHAM kids ($P \leq 0.05$). Skin temperatures of CLOV kids were not different from SHAM kids for 72 h post-treatment ($P > 0.10$).

**BW**

There was no effect of treatment on mean BW over the 3 pre-treatment days ($F_{8, 43} = 1.5, P = 0.20$), and mean BW was not different across groups 1 d pre-treatment [$5.1, 5.1, 5.3, 5.2, $ and $5.6 \pm 0.32$ kg (pooled SE of the difference) for SHAM, CAUT, CASP, CRYO, and CLOV kids, respectively; $P > 0.10$]. There was a day effect on mean weight gain over 7 d post-treatment ($F_{6, 128} = 163.8, P < 0.001$). At 2 d post-treatment, all kids had lower weight gains than 1 d post-treatment ($0.28$ and $0.21 \pm 0.029$ kg, respectively; $P \leq 0.05$). There was no evidence that mean weight gain over 7 d post-treatment differed between groups ($F_{24, 124} = 0.8, P = 0.68$; Figure 5). At 2 wk post-treatment, there were no statistically significant differences in mean BW across treatments [$10.6, 10.3, 11.3, 11.2, $ and $11.3 \pm 0.82$ kg (pooled SE of the difference) for SHAM, CAUT, CASP, CRYO, and CLOV kids, respectively; $F_{4, 44} = 0.7, P = 0.58$].

**Healing Descriptions**

Figure 6 displays examples of wound healing for the disbudding treatments over time. On d 1 post-treatment, CAUT wounds were open and deep with bone clearly visible. Erythema of the tissue surrounding the wounds was observed by d 2. After 7 d, an eschar below the outer layer of skin formed. At 6 wk post-treatment, scabs were apparent.

On d 1, CASP wounds were open and wet; the tissue surrounding the wound was deep red. From 2 d onward eschars formed, with large scabs remaining at 6 wk.

![Figure 3. Mean (±maximum SE of the difference) serum haptoglobin concentrations (g/L) over 24 h in goat kids (n = 10/treatment) that were disbudded using caustic paste (CASP), liquid nitrogen (CRYO), clove oil (CLOV), or a cautery iron (CAUT). For the control (SHAM), the horn buds were massaged but not disbudded. Asterisk indicates means that differ from CAUT kid means at $P \leq 0.05$.](image-url)
On d 1, CRYO wounds were closed and dry. However, on d 2 lesions developed and the wounds were red, wet, and open. Scabs formed by 7 d and were still visible 6 wk post-treatment.

On d 1, CLOV wounds were closed and dry. Three of the 10 CLOV kids had inflammation around the upper eye area (not apparent from images), which was visible for up to 2 d post-treatment. Erythema of the tissue

Figure 4. Maximum (±SE of the difference) change in skin temperature (°C) from baseline (immediately before treatment) of goat kids over 24 h pre- and 72 h post-treatment that have been disbudded using caustic paste (CASP), liquid nitrogen (CRYO), clove oil (CLOV), or a cautery iron (CAUT). For the control (SHAM), the horn buds were massaged but not disbudded. Asterisk indicates treatments that differ from SHAM kid means at $P \leq 0.05$.

Figure 5. Mean (±maximum SE of the difference) weight gain (from baseline; kg) over 7 d post-treatment of goats kids (n = 10/treatment) that have been disbudded using caustic paste (CASP), liquid nitrogen (CRYO), clove oil (CLOV), or a cautery iron (CAUT). For the control (SHAM), horn buds were massaged but not disbudded.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 7</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAUT</td>
<td>Open, wet wound with redness and inflammation of surrounding skin</td>
<td>Similar to d 1. Note: silver color produced by antibacterial spray</td>
<td>Closed, dry wound with eschars formed</td>
<td>Healing wound with small scabs present</td>
</tr>
<tr>
<td>CASP</td>
<td>Open, wet wound with redness and inflammation of skin</td>
<td>Closed, dry wound with eschars formed</td>
<td>Similar to d 2</td>
<td>Large scabs present</td>
</tr>
<tr>
<td>CRYO</td>
<td>Closed, dry wound with redness of skin</td>
<td>Open, wet wound with redness of skin</td>
<td>Closed, dry wound with eschars formed</td>
<td>Small scabs present</td>
</tr>
<tr>
<td>CLOV</td>
<td>Closed, dry wound with redness and inflammation of skin</td>
<td>Closed, dry wound with areas of redness and blackened skin</td>
<td>Similar as d 2 with eschars formed</td>
<td>Small scab present. Note scur formation</td>
</tr>
</tbody>
</table>

**Figure 6.** Images of wound healing over 6 wk in goat kids (*n* = 10/treatment) that were disbudded using caustic paste (CASP), liquid nitrogen (CRYO), clove oil (CLOV), and a cautery iron (CAUT). Sham-handled kids have not been included. Color version available online.
surrounding the site of injection was observed, with some blackened areas at 2 d post-treatment. At 6 wk, small scabs were present on the healed skin.

Scurs (partial regrowth of horns) were not observed in CAUT kids; however, from approximately 3 wk after treatment, scurs grew on 6/10 CASP kids, 8/10 CRYO kids, and 9/10 CLOV kids. Our study was not designed to be an efficacy study.

DISCUSSION

We evaluated 3 alternatives to cautery disbudding (the application of caustic paste, liquid nitrogen, and clove oil) of goat kids using physiological measures of immediate and longer-term pain. The elevated cortisol levels of kids disbudded with caustic paste suggest that this treatment caused more pain than the other disbudding methods for up to 1 h post-treatment. Similarly, researchers studying calves have reported that animals disbudded with caustic paste had higher cortisol concentrations 1 h post-treatment than those that were cautery disbudded (Morisse et al., 1995; Stilwell et al., 2009). In contrast, Vickers et al. (2005) suggested that reductions in behavioral responsiveness of calves disbudded with caustic paste relative to those that were cautery disbudded indicated that caustic paste was less painful; however, these calves were sedated with xylazine, which may have prevented the initial expression of pain-related behaviors. Moreover, cortisol concentrations peaked at 425 nmol/L after an adrenocorticotropic hormone challenge was performed on 1-wk-old goat kids to stimulate a maximal cortisol response (our unpublished data). This response was approximately 55% greater than the response elicited in CASP kids, which peaked at 194 nmol/L, suggesting that although the cortisol response to caustic paste was large, it was not maximal. However, the cortisol response to caustic paste was markedly larger than the response elicited by cautery disbudding.

Cortisol concentrations were higher in CRYO kids than in CAUT kids, which indicates that the application of liquid nitrogen caused more acute pain than cautery disbudding. Calves experiencing cryosurgical disbudding elicit defensive behaviors during the procedure such as struggling, with movements of the head and legs as well as vocalizations (Bengtsson et al., 1996), suggesting that the procedure caused pain. However, calves that underwent cryosurgical disbudding also spent more time lying than cautery disbudded calves, which may reflect greater comfort and perhaps less pain (Stewart et al., 2014). Interpretation of lying behavior is difficult, as increased lying times (compared with controls) may reflect pain in some species but not others; for example, castration of young pigs caused increased lying time compared with intact controls, which suggests that greater pain was experienced (MGlone et al., 1993). Further research is required to evaluate the pain responses of goat kids to cryosurgical disbudding.

The cortisol response of CLOV kids was not different from that of CAUT kids over the 24 h post-treatment period, which indicates that clove oil caused a similar amount of pain as the application of the cautery iron. It was unclear whether elevated cortisol concentrations were in response to damage caused by the needle, the displacement of tissues by the clove oil, or the action of clove oil on the cells. Currently, little research exists on the use of clove oil for disbudding. To our knowledge only 2 efficacy studies exist—1 in calves (Molaei et al., 2014) and 1 in kids (Molaei et al., 2015). These studies reported 100% success in preventing horn growth but did not assess the pain response associated with injecting clove oil. Moreover, the time course of pain associated with the injection of clove oil is unknown; animals treated with clove oil may experience pain for longer than those that are cautery disbudded. The cautery iron instantly destroys nociceptors, whereas eugenol (the main component of clove oil) can cause cellular necrosis up to 1 h after treatment (Kozam and Mantell, 1978). However, clove oil may cause less pain than cautery disbudding as eugenol has similar properties to local anesthesia (i.e., a loss of feeling or sensation within a given area); for example, eugenol can block conduction of action potentials of nerves, reduce synaptic transmission at the neuromuscular joint, and inhibit nerve activity (Markowitz et al., 1992). However, the appropriate dose to induce anesthetic effects is unknown. In the future, pain responsiveness to the injection of clove oil over a 24-h post-treatment period should be measured more intensively. In our study, more blood samples were not taken to avoid affecting kid behavior (measured as part of a companion study).

The increase in cortisol in CAUT kids was larger than that in SHAM kids, which indicates that cautery disbudding causes acute pain. An increase in cortisol post-disbudding compared with sham-handled controls has previously been reported for kids (Alvarez and Gutierrez, 2010; Alvarez et al., 2015; Hempstead et al., 2018) and calves (Graf and Senn, 1999; Grundahl-Nielsen et al., 1999; Heinrich et al., 2009). In the present study, the geometric mean serum cortisol concentrations of CAUT kids were not significantly higher than those of SHAM kids 15 min post-treatment. Large individual variation may explain the lack of significant differences between CAUT and SHAM kids; considerable individual variation was previously reported in a similar...
kid study (Ingvast-Larsson et al., 2011). These earlier results, together with the present study, suggest that cautery disbudded kids experienced more pain than those exposed to handling alone.

The acute pain response for all disbudding treatments appeared to be relatively short lived, as cortisol concentrations typically returned to baseline levels by 1 h post-treatment; in calves, cortisol can remain elevated for up to 4 h post-treatment (Graf and Senn, 1999; Grondahl-Nielsen et al., 1999; Heinrich et al., 2010). Interestingly, the cortisol concentrations of SHAM kids were higher than baseline concentrations at 2 h post-treatment; this was unlikely to be associated with handling, as cortisol concentrations were not elevated within the hour following treatment, when most of the handling occurred. Elevated cortisol concentrations may have been associated with a higher motivation to feed compared with the disbudded groups, as dairy cattle that anticipated feed showed increases in plasma cortisol concentrations (Willett and Erb, 1972).

Both haptoglobin and skin temperature (measured using infrared thermography) can detect inflammatory responses, which are usually associated with pain (Schwartzkopf-Genswein and Stookey, 1997; Ballou et al., 2013). In the present study, a small increase in haptoglobin concentrations of CLOV kids immediately post-treatment and the large increase in haptoglobin at 24 h post-treatment suggest that clove oil can cause inflammation over this time. Interestingly, CLOV kids showed no increase in skin temperature post-treatment compared with SHAM kids, and heat is usually associated with inflammation (Palmer, 1981; Schwartzkopf-Genswein and Stookey, 1997). Changes in temperature may not have been detected because the skin of CLOV kids was intact, whereas the other treatments resulted in broken skin, which may affect heat loss (Stewart et al., 2005). It is important to note the limitations of each measure of inflammation; infrared thermography should be used on skin that is free from dirt, moisture, or foreign material because these can alter emissivity and conductivity and increase local heat loss to the environment (Palmer, 1981). Haptoglobin can be produced in response to systemic disease states (González et al., 2008; Olumee-Shabon et al., 2013); however, changes in haptoglobin concentrations may not be sensitive enough to measure injury to localized soft tissue.

Normal haptoglobin concentrations for adult goats range between 0.39 and 1.26 g/L (Heller and Johns, 2015; Saidu et al., 2016), which can increase to 1.70 g/L with mastitis (Simplicio et al., 2017), which causes inflammation of the mammary tissues and is considered to be painful (Leslie and Petersson-Wolfe, 2012; Fitzpatrick et al., 2013). The values we observed following clove oil injections were substantial, highlighting potentially undesirable side effects. However, pain associated with these inflammatory responses may be alleviated by analgesics such as NSAID (e.g., meloxicam). One CAUT kid excluded from the trial 2 h post-treatment due to a leg joint injury had haptoglobin concentrations of 9.72 g/L; this value was more than 5 times higher than the values observed for CLOV kids. Haptoglobin concentrations have been shown to increase in response to surgical castration in 3-mo-old bull calves but not in dehorned calves (Ballou et al., 2013) or dairy cows with mastitis (Grönlund et al., 2005); this highlights that more sensitive measures of inflammation are required to fully evaluate the inflammatory response associated with different methods of disbudding.

Up to 72 h post-treatment, CASP kids had warmer skin surrounding the horn buds than SHAM kids, indicating that tissue damage and associated inflammation occurred over this time. The skin was warmer on CAUT kids than on SHAM kids 72 h post-treatment, suggesting that inflammation and associated pain may worsen over time for these disbudding methods. It appears that CASP, CRYO, and CAUT kids had burns of similar severity at 72 h post-treatment. Skin surface temperatures were higher in cattle that were heat branded rather than freeze branded for up to 144 h following branding, which may reflect burn severity (Schwartzkopf-Genswein and Stookey, 1997). From our results, it is apparent that some inflammation is caused by all disbudding methods. Segregation between the acute stress response and the inflammatory response to dehorning in cattle was clearly shown by McMeekan et al. (1998). Inflammatory pain can be reduced by NSAID in cauterized disbudded calves (Faulkner and Weary, 2000; Milligan et al., 2004) and goat kids (Ingvast-Larsson et al., 2011; Hempstead et al., 2018). Accumulated research clearly emphasizes the importance of providing animals with NSAID to reduce post-operative pain associated with inflammation.

Local anesthesia can reduce the acute response of calves to cautery disbudding (Graf and Senn, 1999; Grondahl-Nielsen et al., 1999); however, local anesthesia does not appear to be effective for kids. The cortisol response of kids administered local anesthesia before disbudding was similar to that of kids disbudded without anesthesia (Alvarez et al., 2009, 2015). The horn buds of calves are innervated by the lacrimal nerve, whereas in goats the cornual branches of 2 nerves (lacrimal and infratrochlear) innervate the horn buds (Dugdale, 2011); therefore, it may be more difficult to achieve an effective block in goat kids. Because local anesthesia, as administered, appears to be ineffective at reducing pain in goat kids, there is a need to evaluate
alternatives that are less painful than cautery disbudding.

Comparing the degree of pain associated with the different disbudding methods evaluated in this study can be difficult because the mechanism of tissue destruction differed. The severe heat and physical removal of tissue associated with cautery disbudding instantly destroyed nociceptors usually associated with intense acute pain; for this reason, there may be less post-operative pain compared with other methods due to reduced sensation. Immediate pain associated with caustic paste may be lower than the other methods due to the time required for the paste to penetrate the dermis; however, longer-term pain may be more intense than the other methods due to the corrosive action of the paste lasting for as long as it is in contact with the skin (Palao et al., 2007). We hypothesized that kids would have a lower pain response to cryosurgical than cautery disbudding due to the response observed in calves disbudded with liquid nitrogen, which appeared to experience less pain compared with a cautery iron (Bengtsson et al., 1996). A difference in the time required to complete the technique (i.e., 10 s for CRYO and 5 to 7 s for CAUT) may have affected the level of pain experienced. Based on histological results of an earlier pilot study (our unpublished data), 10 s of liquid nitrogen spray was sufficient to destroy horn bud cells. Clove oil can be cytotoxic to human skin cells, causing membrane lysis and consequent necrosis or cell apoptosis (Prashar et al., 2006). Clove oil can also inhibit certain cellular enzymes involved in cell transport processes (Kreydiyyeh et al., 2000), which may cause cell death. However, clove oil has beneficial properties such as anesthetic and anti-inflammatory actions (Markowitz et al., 1992). Further research is necessary to better understand the effect of clove oil on horn bud tissue and associated pain.

All disbudding methods except for clove oil caused burns to the horn buds and surrounding tissue; however, resultant wounds and characteristics of wound healing were quite different among methods. Cautery and caustic paste disbudding appeared to generate more tissue damage than the other 2 disbudding methods. Cautery disbudding caused deep dermal or full thickness burns, which extended through all layers of the skin (Benson et al., 2006; Papp, 2012). Thermal injury denatures surrounding extracellular proteins and causes instant cell death. Circulation is ceased imme-

mediately, which leads to decreased tissue perfusion, both of which prolong recovery (Papp, 2012). Furthermore, the horn buds were removed, resulting in subcutaneous damage (an 18-mm open wound; Wright et al., 1983), which increases not only time required for the stages of healing (inflammation, proliferation, and remodeling) but the risk of infection (Zielins et al., 2015). Bacteria and bacterial byproducts can delay wound healing by disrupting healing processes (Robson, 1997). At 6 wk post-treatment, CAUT kids still displayed scabs, indicating prolonged healing.

The corrosive action of caustic paste causes cellular dehydration and consequent liquefaction necrosis of tissue that usually produces a soft eschar (Palao et al., 2010). The resultant chemical burns are typically superficial to mid-dermal burns characterized by blistering, which can erupt, revealing a red, shiny, moist, and painful wound bed (Papp, 2012). Kids treated with caustic paste had a larger area of tissue damage than CAUT kids, with associated eschar formations that were apparent for up to 6 wk post-treatment. It is difficult to control the spread of caustic paste as it can smear easily; animals may rub against others or scratch the horn bud area with the hind foot (Smith and Sherman, 2009).

Cryosurgical disbudding causes freezing of the tissues and produces intracellular ice and cell dehydration (Gage et al., 1982; Krunic and Marini, 2015). Similar to caustic burns, cryosurgical disbudding causes superficial dermal burns, resulting in erythema and edema. Although cryosurgical disbudding does not initially cause broken skin, consequent blistering and ulcerations were apparent for at least 4 CRYO kids. Broken skin increases the risk of infection, which can delay wound healing (Robson, 1997). Wounds appeared similar to those of calves experiencing cryosurgical disbudding, which displayed vesicle formations (Bengtsson et al., 1996). Cryosurgical disbudding resulted in smaller scabs than caustic paste 6 wk post-treatment.

Clove oil can be cytotoxic in isolated cells (Prashar et al., 2006); however, the mechanisms of wound healing after injection of clove oil into tissue are not well understood. Clove oil caused patches of blackened skin around the injection site (which was likely necrotic tissue) but no open wounds over the 6-wk observation period; this suggests that disbudding with clove oil may allow more efficient healing, potentially reducing the occurrence of infection and damage to the skull and brain caused by the cautery iron (Wright et al., 1983; Thompson et al., 2005). Clove oil injection generated smaller scabs than the other disbudding methods, suggesting that less tissue damage occurred around the horn buds. Further research is required to better under-
stand the mechanisms of cell damage and consequent wound repair after injection of clove oil into the horn buds of goat kids.

Three of the 10 CLOV kids had inflammation of the eye lid (and surrounding area) and haptoglobin concentrations twice their baseline levels, which lasted approximately 24 h post-treatment; incorrect placement of the needle and consequent movement of clove oil closer to the upper eyelid area may explain this result. From our monitoring of wound characteristics, caustic paste and cautery disbudding appear to generate the most tissue damage (and a prolonged healing phase), followed by cryosurgical disbudding. Disbudding with clove oil resulted in less tissue damage and appeared to lead to earlier tissue repair than the other treatments; therefore, we suggest that clove oil shows the most promise as an alternative to cautery disbudding.

Bates et al. (2016) reported that calves disbudded without pain relief had slower growth rates than calves disbudded with pain relief. In the present study, there were no differences in growth rates across treatments over 7 d post-treatment. However, there was lower weight gain 2 d after treatment for all kids, suggesting that handling stress (e.g., blood sampling, restraint) can also affect weight gain. A potential explanation may be a lack of motivation to feed or that feeding was disrupted by blood sampling and associated handling. We ensured that milk feeders were removed from the pens at least 12 h before BW measurements were taken to reduce the effect of gut fill. From our results, it appears that none of the disbudding methods evaluated in this study had a negative effect on the growth rates of goat kids.

Several CLOV, CASP, and CRYO kids developed scurs in our study (no CAUT kids developed scurs as the technique destroys and removes the horn buds); scurs in kids exposed to the alternative disbudding methods were likely due to the use of older animals with mature horn bud cells. Studies using younger animals (as young as 5 d old) to assess the effectiveness of cryosurgical disbudding and clove oil injection reported greater success in preventing scurs (Bengtsson et al., 1996; Molaei et al., 2015). As our study was intended to measure the acute pain response, older animals were required due to high variability of serum cortisol of young goat kids (Chen et al., 1999). Future research should assess how clove oil volume, eugenol concentration, injection technique, and kid age affect scur growth.

CONCLUSIONS

Based on our results, disbudding goat kids with caustic paste is more painful and causes greater tissue damage than the other disbudding methods we assessed. Cryosurgical disbudding appears to cause intermediate pain (i.e., between caustic paste and clove oil) and moderate wounds; however, this method may not be practical due to difficulties associated with managing liquid nitrogen on farm. Clove oil may serve as a valuable alternative to cautery disbudding because it appears to generate a similar acute pain response as cautery disbudding but results in less tissue damage. Future research is required to evaluate the behavioral responses of goat kids experiencing different methods of disbudding; in addition, research is required to validate the efficacy of clove oil disbudding in preventing horn regrowth in dairy goat kids.

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