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Comparison of alternative methods for thermal disbudding in calves

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ABSTRACT

Calves are frequently disbudded to reduce the number of injuries due to horns to other animals and farm workers. The most commonly used method is thermal disbudding which is increasingly causing animal welfare concerns (Stafford and Mellor, 2011; Sutherland et al., 2013, Cozzi et al., 2015). The objective of our study was to evaluate alternative disbudding methods either by injection of clove oil or its synthetic analogue isoeugenol.

Forty Simmental calves (26 male, 14 female) aged between 1 and 5 days were treated using 4 different methods (n = 10): injection of 1.5 mL clove oil, injection of 1.5 mL isoeugenol, injection of 1.5 mL isotonic NaCl solution (control group) and thermal disbudding. For thermal disbudding sedation and local anesthesia were legally required. Horn growth of all calves was repeatedly measured. Computer tomography (CT) of the horn bud region and histological examination of biopsy samples taken from the horn bud region were performed. Additionally, saliva cortisol concentrations were measured before and after intervention.

Significant differences in horn growth were found between isoeugenol treatment and control group ($P = 0.001$), between clove oil treatment and control group ($P = 0.001$) and between thermal dehorning and control group ($P < 0.001$). After clove oil injection 10/10 calves showed signs of inflammation and swelling of the horn bud region including the upper eye lid area. CT images of animals after thermal disbudding showed complete destruction on the horn buds but also severe local damage to the frontal bone. CT images of animals after clove oil or isoeugenol injection showed complete or partial destruction of the horn bud. Histological examination was performed at 1 horn bud per animal of 5 per each group. Eight biopsy samples showed normal unchanged epidermis and dermis (3 isoeugenol, 5 control group), in 6 samples normal vital skin side by side with necrotic epidermis and/or dermis was found (2 isoeugenol, 2 clove oil, 3 thermal dehorning). In one sample (thermal dehorning) necrotic bone with osteomyelitis was detected. In 3 samples (thermal dehorning) dystrophic calcification was present. After clove oil treatment necrotic epidermis and dermis as well vital skin with a purulent crust was detected.

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The highest mean cortisol value was measured in calves treated with clove oil. Fifteen minutes after treatment the mean value of thermal dehorned calves showed the lowest values, however these calves were sedated for the treatment.

Thermal disbudding tended to have the highest efficacy of disbudding. Horn growth was also substantially decreased in clove oil and in isoeugenol group. In cases in which the horn growth was not totally suppressed the horns remained very small. Based on our results, disbudding calves with isoeugenol causes less tissue damage than thermal disbudding. Since clove oil caused temporary swelling we consider it less suitable. Future research is required to evaluate the behavioral responses of calves experiencing these methods of disbudding; in addition, more research is needed to optimize the injection volume and technique in a larger group of animal.

Introduction

Thermal disbudding is a commonly performed procedure in calves to prevent horn growth and subsequently injuries to people and other animals. The most common method for disbudding calves is the application of a hot iron (Stafford and Mellor, 2011; Cozzi et al., 2015). Thermal disbudding causes long and short-term pain, slowly healing thermal wounds and wound infections and additionally an increased plasma cortisol concentration (Graf and Senn, 1999; Grøndahl-Nielsen et al., 1999; Faulkner and Weary, 2000; Sutherland et al., 2013). The total plasma concentration rises immediately, peaking after about 30 min. The maximum plasma cortisol concentrations occurred between 0.5 and 1.0 h after treatment. The highest mean cortisol concentrations were maintained between 1.5 and 3 h after intervention and then declined rapidly to plateau values and.then decreases to a plateau which persists for 5-6 h before returning to pretreatment levels. (Sylvester et al., 1998a,b). Necrosis and meningoencephalitis have been described as further complications (Nation and Calder, 1985). Due to animal welfare concerns, research is focusses on alternative disbudding methods. Beside of breeding genetic hornless animals, the injection of clove oil or isoeugenol into the horn bud has been described as an alternative method preventing horn growth in calves (Molaei et al., 2014; Sutherland et al., 2019a; Sutherland et al., 2019b; Schoiswohl et al., 2020). Clove oil and the synthetic analog isoeugenol cause cell necrosis but also result in local analgesia (Neiffer and Stamper, 2009). In low concentrations clove oil also has anti-inflammatory and anti-oxidative effects (Neiffer and Stamper, 2009).

To the authors knowledge 2 reports describe the successful use of clove oil or isoeugenol to prevent horn growth (Molaei et al., 2014; Schoiswohl et al., 2020), while others reported only incomplete suppression of horn growth (Hempstead et al., 2018b; Sutherland et al., 2019a,b). Molaei et al. (2014) observed no horn growth after injection of 0.5 mL clove oil into the horn bud of calves. In contrast, Schoiswohl et al. (2020) reported complete suppression of horn growth after injection of a higher dosage (1.5 mL clove oil or isoeugenol) into the horn bud region); the suppression was incomplete after injection of 0.5 mL. Schoiswohl et al. (2020) found that success rate depends on dosage but also on the age of animals because in younger calves the horn bud is not or less attached to the bony skull calotte.

The present study was performed to evaluate if the injection of clove oil and the injection of isoeugenol as alternative disbudding methods are equally effective but cause less tissue damage and pain than thermal disbudding. This study includes measurements of horn growth, computer tomography (CT) of the horn bud region, histological examination of biopsy samples taken from the horn bud region and saliva cortisol concentration before and after treatment. For this study 2 hypotheses were tested.

1. Clove oil and isoeugenol injections are equally effective in the suppression of horn growth in comparison to thermal disbudding, but cause less pain and distress.
2. The alternative treatments result in less severe tissue damage than thermal disbudding and are followed by faster wound healing.

Materials and methods

Animals

The study was conducted in 40 Simmental calves (26 male, 14 female) aged 1-5 days (mean 2.88, SD 1.29 d). The study was approved by the institutional ethics and animal welfare committee and by the governmental animal welfare body in accordance with good scientific practice guidelines and national legislation (GZ 68.205/0049-WF/V/3b/2016). All animals were housed at a dairy farm in Lower Austria. Calves were weighed and identified by individual ear tags. All calves were assigned at random by drawing lots to one of the 4 groups (10 calves per group). Both horn buds of each animal were treated with the same method.

Groups

Control group: calves were injected with 1.5 mL of NaCl (Kochsalz "Braun 0.9% - Infusionslösung", B. Braun Austria GmbH, Austria) as used by Schoiswohl et al., 2020.

Thermal dehorning group: calves were thermally disbudded using a hot iron (Buddex, Albert Kerbl GmbH, Germany) during sedation (Sedaxylan®, 20mg/ml, Eurovet Animal Health B.V, Netherland, xylazine 0.1 mg/kg bodyweight i. m.) and local anesthesia in area *Ramus cornualis* of *Nervus zygomaticus* (8 mL Procamidor®, 20 mg/mL, Richter Pharma AG, Austria, procaine hydrochloride per each horn bud local). Horn buds were removed after the hot iron had been applied and rotated. After intervention, wounds were treated locally with a CTC (Chlortetracycline)-Spray (Cyclo-spray®, Dechra Veterinary Products GmbH, Austria).

Clove oil group: calves were injected with 1.5 mL (as used by Schoiswohl et al., 2020) of clove oil (*Syzygium aromaticum*; synonym: *Eugenia cariophylata*; Herba Chemosan Apotheker-AG, Austria) from lateral into the center of each horn bud at a 45° angle between nasal bridge and ear using a 16 G needle (BOVIVET 16 G x 1-1/2″ 1.6 × 38 mm, Jørgen KRUUSE A/S, Denmark) (Figure 1).

Isoeugenol group: calves were injected with 1.5 mL (as used by Schoiswohl et al., 2020) of isoeugenol ($C_{10}H_{12}O_2$; 2-methoxy-4-prop-1-enylphenol) Merck KGaA, Germany) applying the same injection procedure like group clove oil.

All Animals were treated by the same person (JS = veterinarian).

Figure 1. Technique of injection from lateral into the center of each horn bud at a 45° angle between nasal bridge and ear using a 16 G needle.

Measurement of horn growth

Calves were observed concerning horn growth over a time period of 9 months. Horn growth (length in mm) was measured 4 times (1, 2, 4 and 9 month after treatment) using a gauging tool.

Computer tomography (CT) of the horn bud region

Computed tomography of the head in twenty calves (5 per group) was performed using a 16-slice scanner (SOMATOM® Emotion 16, Siemens Healthcare, Erlangen, Germany) 21d after treatment. Animals were anaesthetized and placed in sternal recumbency. Technical settings were 130 kV and 240 mAs. Effective slice thickness was 0.5 mm. Collimation was 9.6×0.6 mm. The scans were reconstructed using both soft tissue and bone algorithms. Early (arterial), early venous (120 seconds post injection) and late venous (180 seconds post injection) contrast series (600 mg iodine per kg body weight intravenously, 1.5 mL/s) were performed in all cases. CT images were stored in a picture archiving and communication system. Multiplanar reconstructions and surface models were calculated using a syngo MultiModality Workplace (JiveX; Visus Health IT GmbH, Bochum, Germany).

CT images were reviewed without knowledge of the treatment method by 1 observer (S.K.). Observations regarding presence or absence of the horn bud, or intervention-correlated soft or bone tissue changes were noted.

Animals for CT investigation were selected by random function of Excel®. The calves were consecutively numbered in their groups. Numbers drawn by the computer program in a groupwise manner ensured that each calf in the groups had the same chance to be selected for CT investigation.

Histological examination of biopsy of horn bud region

Biopsy samples of the horn bud region were sampled from 20 calves (1 horn bud per animal, 5 per each group) 21 days after treatment. Punch biopsy samples (x mm) were obtained in calves placed under general anesthesia and after pain medication (Carprodolor®, 50mg/ml, Virbac AG, Switzerland, 1.4 mg Carprofen/kg bodyweight; s. c.) was administrated.

Biopsy samples were fixed in 10% neutral buffered formalin and if necessary decalcified (Decal®, StatLab, McKinney, Texas, USA) before embedding in paraffin-wax. Samples were sectioned at 2 µm and stained with hematoxylin and eosin (HE) for the histopathological examination. The biopsy samples were examined in the order they were taken, the examiner (DT) was also not aware of the treatment.

Animals for histological examination were selected by random function of Excel®.

Saliva sampling – cortisol measurement

After clinical examination saliva from each calf were collected pre and post treatment with a salivette (Salivette® Cortisol, Sarstedt AG and Co, KG Germany) fixed at a clamp. Sampling was performed immediately before treatment and 15 minutes and 60 minutes after treatment. All salivettes were stored frozen (-80°C) until analysis.

Saliva samples were thawed in a water bath at 38°C for 20 minutes and afterwards centrifuged at 2500 g for 10 minutes. After centrifugation 50 µL of each sample was transferred to Biorad tubes and 450 µL assay buffer added. Aliquots were then analysed with a cortisol enzyme immunoassay (Palme and Möstl, 1997), which has already been used in calves' saliva (Wagner et al., 2013). The laboratory was blinded to the used disbudding method.

Statistical analysis

The horn buds are considered the experimental units resulting in a sample size of 20 horn buds per group .Descriptive and comparative statistics were calculated using Microsoft® Excel 2010 and IBM SPSS Statistics (Version 24.0). Comparisons between different methods outcome variables were performed by Kruskall-Wallis-Test (for comparisons of multiple methods) and Wilcoxon-Mann-Whitney U test (for comparisons of 2 methods). For all analyses, a P-value < 0.05 (5%) was considered as significant.

Results

Horn growth

Horn growth is shown in Table 1 (horn growth 9 month after clove oil and isoeugenol injection) and Figure 2 (horn growth 9 month after intervention compared between the 4 different groups).

Nine months after disbudding horns of control group were grown between 40 mm and 89 mm, in thermal disbudding group there was no horn growth. The animals of clove oil group showed a horn growth between 0 mm and 54 mm and of isoeugenol group between 0 mm and 53 mm.

In the clove oil group, 9/20 horns (45%) showed no horn growth, 5/20 (25%) horns showed slight horn (< 10 mm) growth, 5/20 (25%) horns showed moderate (20-40 mm) horn growth and 1/20 (5%) horns showed unimpaired (> 40 mm) horn growth.

In the isoeugenol group, 7/20 (35%) horns showed no horn growth, 6/20 (30%) horns showed slight horn (< 10 mm) growth,

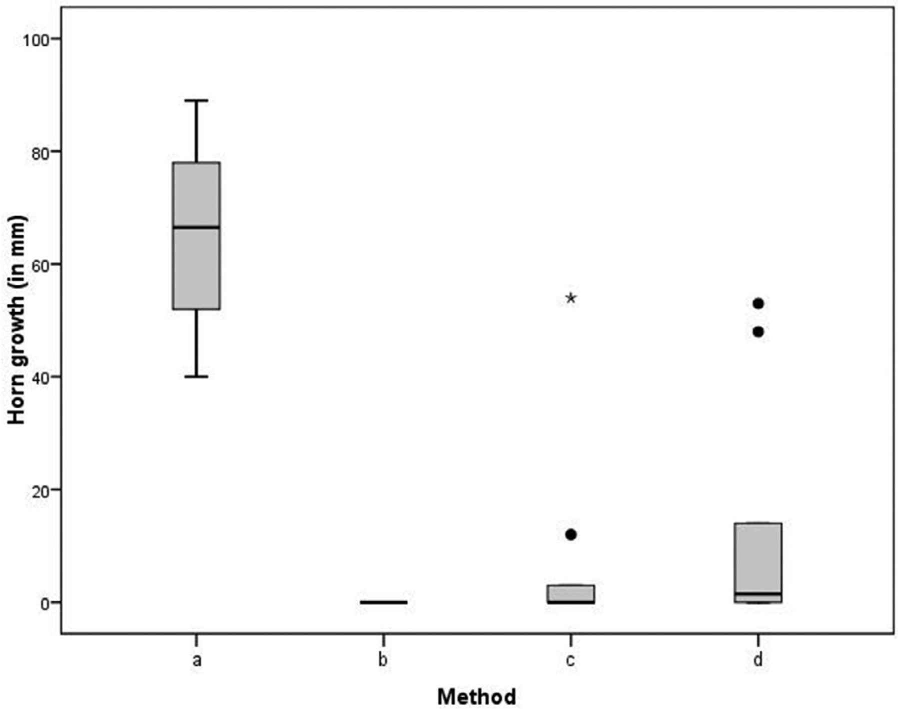

Figure 2. Horn growth (cm) 9 month after intervention in millimeters compared between the different groups: a-control group (NaCl); b-thermal disbudding; c-clove oil; d-isoeugenol; middle line = median, filled circles represent outliers between 1.5. and 3.0 times the interquartile range, asterisks indicate outliers more the 3.0 times the interquartile range.

Table 1
Evaluation of the horn growth 9 months after the injection of clove oil (CO) and isoeugenol (IE); 0 mm=no horn growth, 0-10 mm=slight horn growth, 10-20 mm=moderate horn growth, more than 20 mm=unimpaired horn growth.

Calf/Sex	Substance	Horn left	Horn right
1 / f	CO	No horn growth	No horn growth
4 / m	CO	Moderate horn growth	Moderate horn growth
6 / m	CO	Moderate horn growth	No horn growth
8 / m	CO	Slight horn growth	Slight horn growth
9 / f	CO	Slight horn growth	No horn growth
11 / m	CO	Moderate horn growth	Unimpared horn growth
15 / m	CO	Moderate horn growth	Slight horn growth
17 / m	CO	No horn growth	No horn growth
19 / m	CO	Slight horn growth	No horn growth
20 / f	CO	No horn growth	No horn growth
2 / m	IE	Unimpared horn growth	Unimpared horn growth
3 / m	IE	Slight horn growth	Slight horn growth
5 / m	IE	Slight horn growth	Moderate horn growth
7 / f	IE	No horn growth	No horn growth
10 / f	IE	Slight horn growth	Slight horn growth
12 / m	IE	Moderate horn growth	No horn growth
13 / f	IE	Moderate horn growth	No horn growth
14 / f	IE	Unimpared horn growth	Unimpared horn growth
16 / m	IE	Slight horn growth	No horn growth
18 / m	IE	No horn growth	No horn growth

3/20 (15%) horns showed moderate (20-40 mm) horn growth and 4/20 (20%) horns showed unimpaired (> 40 mm) horn growth.

In the control group, all calves showed an unimpaired horn growth.

After thermal disbudding, 20/20 horns did not grow.

In female calves, 8/14 horns (57.1%) (5/6 clove oil [83.3%] and 3/8 isoeugenol [37.5%]) showed no horn growth. In male calves, 8/26 horns (30%) (4/14 clove oil [28.6%], 4/12 isoeugenol [33.3%]) showed no horn growth.

Significant differences in horn growth were found between isoeugenol treatment and control group ($P = 0.001$), between clove oil and control group ($P = 0.001$) and between thermal dehorning and control group ($P < 0.001$). There was no significant difference between clove oil treatment and thermal disbudding ($P = 0.143$) and between isoeugenol treatment and thermal disbudding ($P = 0.063$). No scurs were observed (Figure 2).

After clove oil injection all calves showed signs of inflammation (swelling) of the upper eye lid area below the horn bud region. All 10 calves remained at normal general behavior and good appetite. Swelling started within hours after clove oil injection, was worst 1 day after injection and decreased after a treatment with 0.06 mg dexamethasone/kg bodyweight (Dexa "Vana"® 2 mg/ml, VANA GmbH, Austria) once.

CT changes of the horn bud region

CT images in 5 control animals (NaCl) were diagnosed as normal and showed similar developmental stage of their horn buds without any changes in horn bud region. CT images of animals after thermic intervention revealed complete destruction of the horn bud area and severe local destruction of the frontal bone in 5 animals (10 horn buds). Moreover, necrotic bone material (less bone substance with increased density) was detectable. The CT images of animals after clove oil injection showed partial destruction of the horn bud or malformation in 5/5 animals (8 horn buds). In 2 horn buds the architecture was totally destroyed. CT images of animals after isoegenol injection showed partial destruction of the horn bud in 5/5 animals (8 horn buds), whereas the architecture was completely destroyed in 2 horn buds.

Histological examination of biopsy samples from the horn bud region

In total 20 biopsy samples were pathohistologically examined, in 2 cases (thermal dehorning and isoeugenol group) a decalcifi-

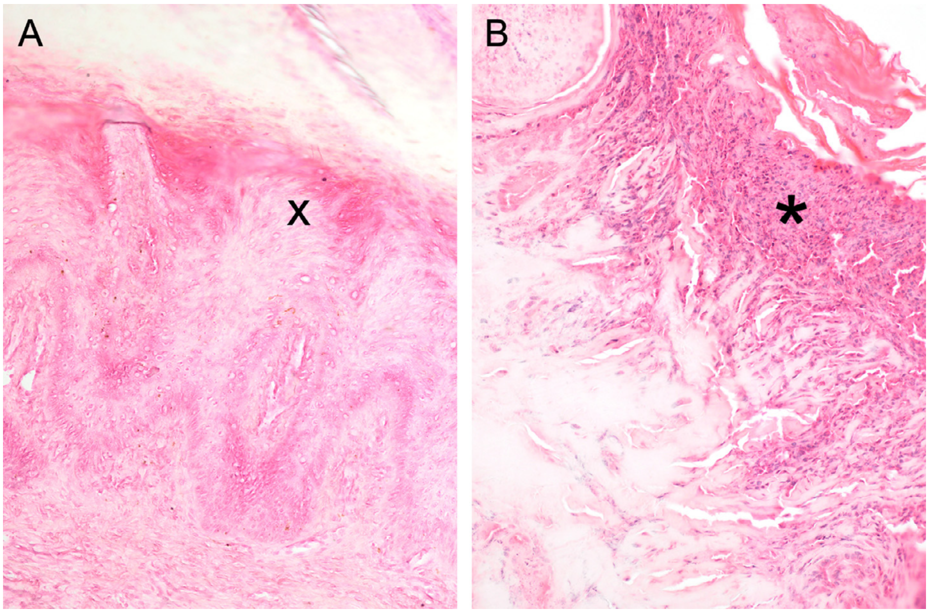

Figure 3. (A) unchanged skin sample with hyperplastic epidermis (x) and keratinized epithelium representing the horn bud. (B) necrotic epidermis with a severe infiltration with degenerated neutrophils (asterisk).

cation was necessary before examination. All 20 samples consisted of epidermis and dermis. Eight samples out of 2 groups (5 control group, 3 isoeugenol) showed normal unchanged skin with hyperplastic epidermis representing the horn bud. The remaining 12 samples showed a varying degree of purulent inflammation and/or necrosis affecting epidermis and dermis in 5 cases (3 clove oil, 2 isoeugenol) (Figure 3) and exclusively in the epidermis in 4 cases (1 clove oil, 3 isoeugenol). In the thermal dehorning group, 1 sample showed not only necrotic skin but also necrotic bone tissue with purulent osteomyelitis. Within the clove oil group 2 samples showed only focal epithelial changes in terms of a focal purulent inflammation with necrosis and a purulent crust respectively. In the thermal dehorning group in 4 out of 5 samples, proliferation of fibroblasts or granulation tissue was visible in the dermis accompanied by a secondary dystrophic calcification in 2 samples. All biopsy samples consisted of epidermis and dermis ensuring a sufficient thickness for evaluation

Salivary cortisol concentration

Cortisol concentrations are shown in Figure 4. One sample (control group) 60 min after injection) was not available. A significant increase of cortisol concentration was observed in animals treated with isoeugenol between the cortisol concentration before treatment and 15 minutes after injection (P = 0.041). Fifteen minutes after treatment the mean value of thermal disbudded calves was the lowest value, followed by control group (NaCl) and isoeugenol. However, it has to be considered that the calves were sedated for thermal disbudding which influences the cortisol secretion. The highest mean cortisol value was measured in calves treated with clove oil. The increase of cortisol level in group clove oil and in group isoeugenol was higher than in control group. Sixty minutes after treatment the highest mean value (4.04 ng/mL) was measured in clove oil group. At this time mean cortisol values were almost the same in thermal dehorned calves (3.69 ng/mL) and calves treated with isoeugenol (3.67 ng/mL) (Figure 4).

Discussion

Currently there is limited research on the use of clove oil and isoeugenol for disbudding in calves. Molaei et al. (2014) and Schoiswohl et al. (2020) described injection of clove oil or isoeugenol for preventing horn growth in calves with high success rate. In addition, Molaei et al. (2015) reported successful application of clove oil in goat kids. However the number of involved animals was low in these studies. Other studies described lower efficacies and asking for further studies for refinement of the technique (i.e., administration methods to improve efficacy) before clove oil injection could be considered an alternative to thermal disbudding (Hempstead et al., 2018a; Hempstead et al., 2018b; Sutherland et al., 2019a; Sutherland et al., 2019b, Still Brooks et al., 2021).

Schoiswohl et al. (2020) reported that the efficacy of clove oil and isoeugenol for disbudding calves is dependent on the dosage and that the calves need to be treated at a very young age (less than 4 days old). In contrast to Molaei et al. (2014 and 2015) and Schoiswohl et al. (2020), Hempstead et al. (2018b) reported that clove oil treatment appeared to be less effective at preventing horn growth than thermal disbudding because of a higher incidence of scurs and unaffected horn growth. In present study no scurs were observed after clove oil and isoeugnol treatment. There was no significant difference in horn growth between isoeugenol treatment and thermal disbudding and clove oil treatment and thermal dehorning.

Independently from the measured horn growth, implications for the practice have to be considered and discussed with farmers. After discussion with farmers and the animal health services, we believe that it is reasonable to consider slight horn (< 10 mm) and moderate (20-40 mm) horn growth still as treatment success as the main aim the prevention of injuries has been achieved.

Clove oil and isoeugenol were applied using a 16G needle to inject laterally into the bud region, whereas thermal disbudding involved pressing a cautery iron directly on the horn bud. Consistent administration of the full volume of clove oil or isoeugenol into the

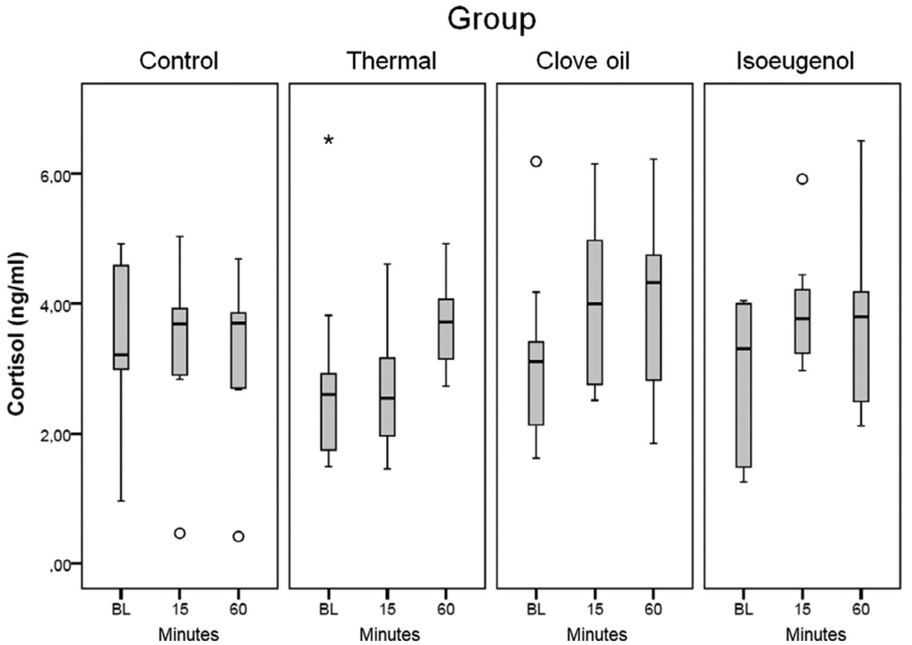

Figure 4. Saliva cortisol concentrations (ng/ml) before (BL) and 2 times after treatment (15 and 60 minutes). Calves of group thermal disbudding were sedated for the treatment; therefore 15 minutes measurements are biased. Significant increase between BL and 15 in group isoeugenol (P = 0.041). Middle line = median, empty circles represent outliers between 1.5. and 3.0 times the interquartile range, asterisks = indicate outliers more the 3.0 times the interquartile range.

center of the horn bud which would be the correct location was sometimes difficult to achieve. Due to insufficient restraint during injection, struggling calves during application or moving the head, the needle may have been moved in a slightly incorrect location. It seems possible that the development of an applicator could improve the injection of a consistent volume at the correct location may improve efficacy (Hempstead et al., 2018b). Incorrect administration or an insufficient volume reached at the center of the horn bud in the epidermis may lead to unaffected horn growth or scurs.

To the best of the authors knowledge there is just one other study which describe CT findings after injection of clove oil and isoeugenol in goat kids (Schoiswohl et al., submitted to Journal of Veterinary Behavior). CT images after thermal disbudding showed complete destruction of the horn buds; however additionally the bone destruction involved parts of the frontal bones in all examined goat kids after thermal disbudding. CT images after clove oil and isoeugenol treatment showed only partial destruction or malformation of horn buds. No involvement of the frontal bones of goat kids was seen after clove oil or isoeugenol treatment (Schoiswohl et al., unpublished data). In the study presented here CT images of the 5 control animals were interpreted as normal and showed similar developmental stage of their horn buds without any changes in horn bud region. CT images of animals after thermic intervention showed complete destruction of the horn system and severe local destruction of the frontal bone in 5 animals (10 horn buds). Necrotic bone material (in form of less substance with increased density) was detectable. Hot iron disbudding - if undertaken too vigorously - may result in thermal damage of the underlying bone, meninges and even the brain (Wright et al., 1983; Thompson et al., 2004). Due to different anatomy of the frontal bones and sinus of kids and calves) the risk for meningitis and damage of the brain seems to be higher in kids. In this study thermal dehorning was performed carefully and according to the manufacture's guidelines, however damage of frontal bones was observed. CT images of animals after clove oil and isoeugenol treatment demonstrated partial destruction or malformation of the frontal bones. If the horn bud epidermis cannot be completely destroyed by clove oil or isoeugenol injection, keratinization of epidermal cells results in growth of horns or scurs. Scurs after thermal disbudding usually result from insufficient burning due to the effort to reduce the risk of injury to the frontal bone and brain (Dawson et al., 2007). Partial destructions after clove oil injection and isoeugenol injection may the reason for incomplete success. Regarding the effectiveness of isoeugenol to prevent horn growth, more studies are needed (Juffinger et al., 2021).

In about 75% of biopsy samples, the transition from hairless skin with hyperplastic epidermis to normal skin with hair follicles was traceable, indicating correctly positioned punch biopsies. Normal unchanged skin was found in all samples belonging to the control group, as expected, but also in 3 out of 5 samples in the isoeugenol group. The reason for this could be a slight change during the positioning of the needle caused by struggling calves, resulting in an uneven or misplaced distribution of the isoeugenol. Necrosis and purulent inflammation in varying degree was detectable in all samples in the clove oil and 2 out of 5 samples in the isoeugenol group. Prashar et al. (2006) and Molaei et al. (2015) have previously demonstrated the cytotoxic effect of clove oil and eugenol in horn buds and in vitro using cell cultures of 3 different human cell types. It is assumed that the cytotoxicity is directed at the cell membrane leading to necrosis or apoptosis, 2 mechanisms that can occur simultaneously and may not be distinguishable microscopically. In the study of Molaei et al. (2015) the necrosis of horn bud was accompanied by a mild infiltration with neutrophils. In contrast to our samples where in some samples a severe neutrophilic reaction was detectable. This could be due to the fact that in our study, samples were taken between 17 and 22 days after the treatment versus 5 and 10 days respectively in the study of Molaei et al. (2015). In the thermal dehorning group remnants of necrotic bone and a purulent osteomyelitis was seen only in one sample, complications that were expectable. Although the CT evaluation showed partial destruction or malformation of the frontal bone after clove oil or isoeugenol administration, no necrotic bone tissue was visible in either group. Proliferation of fibroblasts and signs of granulation tissue – indicating the expected wound healing after about 20 days- was only seen in samples belonging to the thermal dehorning group. Whether this is caused by the fact that with this technique an increased and more widespread pressure to the underlying tissue must be exerted in comparison to the injection of clove oil or isoeugenol, is unclear.

Comparing cortisol concentration of the different groups in this study has to reviewed critically because in thermal disbudding calves sedation had to be used and xylaxine influences cortisol secretion (Hefti, 2010). Administration of non-steroidal-anti-inflammatory drugs also reduced signs of pain significantly, but the higher cost of using such analgesic drugs means they are not regularly used in field practice and so we did not use in presented work (Wagmann et al., 2018). However over all the cortisol concentrations in the treatment groups are not significantly higher than in control group. Despite this, we have some limited indications that, clove oil and isoeugenol causes a stress response 60 minutes after injection. Sutherland et al. (2019b) reported that clove oil injection did not cause any damage of the skull or brain of calves. Sutherland et al. (2019b) described injection clove oil cause less initial pain and did not appear to cause more pain than hot iron dehorning 48 hour after treatment. Furthermore using clove oil for suppression horn growth does not appears to cause more pain and could cause fewer acute or long-term welfare concerns. In Juffinger et al. (2021) results suggest that injection of isoeugenol causes less pain and thus seems to be beneficial compared to thermal dehorning, while clove oil was not advantageous.

In this study we did not use an NSAID before/after thermal disbudding. In literature there are several studies of calves suggesting that the welfare of calves is improved if NSAIDs are used. The cortisol concentration after thermal disbudding might have been lower if we had used NSAIDs.

Animals with signs of inflammation after clove oil injection were also observed from Hempstead et al. (2018b) in goat kids. These observations are very interesting because on the one hand such swelling is not observed in goat kids (Schoiswohl et al., not published data) and calves (Schoiswohl et al., 2020) and, on the other hand, eugenol, the main component of clove oil, has anti-inflammatory properties (Markowitz et al., 1992). Perhaps clove oil is a natural product and so it is not as clear as synthetic isoeugenol and so reactions/inflammation could be caused due to accompanying substances. Injections of clove oil and isoeugenol in goat kids and calves for the purpose of disbudding may lead to the development of mechanical hypersensitivity. In comparison with clove oil, isoeugenol offered the advantage of inducing a more consistent anaesthetic effect and, at least in goats, less sustained hypersensitivity, which resolves 24 h after injection (Frahm et al., 2020). For further research, we would prefer isoeugenol instead of clove oil because no swelling was observed.

According to the results the alternative treatments (especially isoeugenol) result in less severe tissue damage than thermal disbudding and even through more future research is needed to optimize the injection volume and technique we would assume that the use of isoeugenol increase animal welfare. May additionally use of NSAID would further increase animal welfare.

Horn growth was substantially decreased in in clove oil and isoeugenol group and if the horn growth was not totally suppressed the horns remained very small. Based on our results disbudding calves with isoeugenol causes less tissue damage than thermal disbudding. In this study, low number of animals could be seen as a potential limitation. Future research is needed to optimize the injection volume and technique in a larger group of animal and so a field study is in progress to evaluate the success of using isoeugenol in different farms by different local veterinarians.

Acknowledgments

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Conflict of Interest

We have no conflicts of interest to disclose.

Ethical statement

The study was conducted on forty Simmental calves (26 male, 14 female) aged between 1 and 5 days and was approved by the institutional ethics and animal welfare committee of the Veterinary University of Vienna in accordance with good scientific practice guidelines and national legislation (GZ 68.205/0049-WF/V/3b/2016).

We have no conflicts of interest to disclose.

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