Original Article

Assessing the efficacy of eutectic mixture of local anesthesia cream and vapocoolant spray in mitigating the pain associated with ear-tagging in pre-weaned calves

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Abstract

This study evaluated the efficacy of a eutectic mixture of local anesthetics (EMLA) cream and vapocoolant spray (VS) in preventing pain in calves following ear-tagging. Thirty-four calves were randomly assigned to three groups: no anesthesia control (CON, n=11), EMLA applied 60 minutes prior (EMLA, n=11), and five-second VS application immediately prior (VS, n=12). Pain pressure threshold (PPT) was measured at 18±1 days of age, with the left ear of the CON group serving as the sham group (Sham, n=11), followed by ear-tagging at 21±1 days. Post-tagging assessments included pain-related behaviors, serum cortisol levels, heart rate, and wound scores. The PPT values were higher in the EMLA and VS groups than in the CON group ($P < 0.001$), confirming their anesthetic efficacy. The frequencies of head shaking and leg movement were lower in the Sham and EMLA groups compared to the CON group at 0–5 minutes post-tagging ($P < 0.01$). Similarly, tail flicking and leg movement frequencies were significantly lower in the Sham and VS groups than in the CON group. Back arching occurred in 100% of calves in the CON and VS groups but only 9.1% of EMLA-treated calves. Wound healing was similar across groups, with ≥50% of tagged calves scoring 1. No significant differences in heart rate or serum cortisol levels were observed, suggesting their limited reliability as pain indicators. In conclusion, both EMLA cream and VS effectively alleviated pain after ear-tagging, with EMLA providing superior analgesia. Integrating local anesthetics into routine ear-tagging can improve calf welfare.

Keywords: calves, ear-tagging, local anesthetics, pain alleviation

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Introduction

Globally, ear-tagging is a routine practice on farms that plays a crucial role in cattle identification, herd management, and disease tracking (Awad, 2016; Lomax *et al*., 2017). Typically, ear tags record an animal's identification number, facilitating efficient internal herd management. Animal identification is particularly essential in epidemiological investigations of disease outbreaks and transmission. Moreover, increasing public concerns regarding food safety and product traceability have further emphasized the importance of precise livestock identification. Although ear-tagging is known to cause pain in calves, it is generally accepted that local anesthesia is not required when the procedure is performed during the neonatal period (Hayer *et al*., 2022).

From an animal welfare perspective, the administration of appropriate anesthesia or analgesia is recommended before conducting painful procedures. Several published studies have primarily focused on pain mitigation strategies for procedures such as castration in male livestock (Torrey *et al*., 2009), tail docking in pigs (Torrey *et al*., 2009), and disbudding in calves (Winder *et al*., 2017). These studies aimed to prevent post-procedural pain through the use of anesthetics, either alone or in combination with sedatives or analgesics (Liu *et al*., 2023; Nfor *et al*., 2016; Adam *et al*., 2021). However, the routine use of sedatives or anesthetics is often impractical on farms due to the requirement for veterinary intervention, as well as the additional time, labor, and costs associated with their administration, making on-farm implementation challenging (Stilwell *et al*., 2019). Therefore, a simple and safe anesthetic approach is needed to ease challenges faced on farm operations while enhancing animal welfare.

Numerous studies have suggested that ear-tagging may induce pain and distress in a variety of livestock species, including calves (Stewart *et al*., 2013; Schnaider *et al*., 2022), piglets (Numberger *et al*., 2016), kids (Hussein & Hidayet, 2019; Zebaria *et al*., 2021), and lambs (Karakuş & Karakuş, 2017). Nevertheless, the application of medical vapocoolant spray (VS) prior to ear-tagging has been shown to effectively reduce struggling behavior in calves (Lomax *et al*., 2017). After application, the cooling effect of VS lowers the skin surface temperature by approximately 10°C through the release of compressed air, thereby reducing nerve conduction in the underlying tissues and providing a short-term anesthetic effect. This cooling effect provides a short-term analgesic effect by temporarily blocking nerve transmission (Lomax *et al*., 2017). The eutectic mixture of local anesthetics (EMLA) cream, which combines lidocaine and prilocaine, provides analgesia by blocking sodium (Na^+) channels in cell membranes, stabilizing membrane potential, and inhibiting nerve conduction in the treated area. The anesthetic effect may persist as long as the cream is present on the skin (Gajraj *et al*., 1994). Previous studies in human medicine have demonstrated that both EMLA cream and medical VS effectively reduce pain-related responses, including crying and screaming when applied before procedures such as catheterization or intramuscular injection (Cohen and Holubkov, 1997; Dalvandi *et al*., 2017; Mace, 2017). These findings suggest that both methods provided effective short-term pain relief, with the advantages of easy administration and high safety.

This study aims to evaluate the effectiveness of two easily applied local anesthetics, EMLA cream, and medical VS, in alleviating pain and distress in calves after ear-tagging. We hypothesized that calves treated with either local anesthetic would exhibit a lower frequency of pain-related behaviors and minimal changes in cortisol concentrations, indicating anesthetic efficacy following the ear-tagging procedure.

Materials and Methods

Animals: This study was conducted on a dairy farm at the National Chung Hsing University from July 2023 to January 2024, where a total of 120 Holstein dairy cows were housed. Thirty-four healthy female Holstein calves (18 ± 1 day old) were enrolled in the study. The calves were housed individually in pens with slatted floors (125 × 75 × 100 cm), elevated 35 cm above the ground, within an indoor nursery. The calves were provided with milk twice daily and ad libitum access to water. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of National Chung Hsing University (Approval No. 112-059).

Experimental design and procedures: Thirty-four calves (18 ± 1 days old) were randomly assigned to 3 groups for the pain pressure threshold (PPT) test in Stage 1 and later underwent the ear-tagging (ET) procedure in Stage 2 at 21±1 days of age. The groups include the control group (CON, n = 11), EMLA group (EMLA, n=11; ear-tagged with prior EMLA cream application), and the VS groups (VS, n=12; ear-tagged with prior vapocoolant spray application). The experimental procedure is summarized in Figure 1.

In Stage 1, each calf was restrained in a neck clamp for the PPT test, during which pressure was applied directly to the ear using an algometer. Following the method of Heinrich *et al*. (2010), an algometer (FORCE TENTM pain tester, Wagner, USA) equipped with a 1 cm^2 rubber probe was used to measure PPT. For each measurement, pressure was applied to the ear, positioned between the two main cartilages, approximately one-third of the distance from the base of the ear (Figure 2), at a rate of 10 N/sec until the calf exhibited struggling behavior. The maximum tolerated pressure was recorded. Each site was tested five times, and the three most consistent maximum pressure values were used for analysis. All three groups first underwent the initial PPT test on the left ear without anesthetic treatment, followed by the second PPT test on the right ear with or without analgesic treatment. During the first stage, the left ear of calves in the control group served as the sham test (Sham group, n = 11), in which tactile stimulation was performed under the same restraining conditions to assess potential bias in behavioral responses. In the EMLA group, EMLA cream (Lidopin 5% Cream, Panion & BF Biotech Inc., Taiwan) was applied to both the medial and lateral sides of the tagging area, approximately 2 ×

2 cm with a thickness of 0.2 mm, 60 minutes prior to PPT measurement and ear-tagging. In the VS group, a medical vapocoolant spray (Salonpas Icing Spray, Hisamitsu Pharmaceutical Co. Inc., Japan) was applied once for 5 seconds to the inner surface of the treated ear immediately after either the PPT measurement or the ear-tagging procedure to induce local cooling and pain relief.

In Stage 2, the ear-tagging procedure was conducted following the method described by Lomax *et al*. (2017). Calves in all three groups were subjected to the same standardized ear-tagging procedure on the right ear using a commercially available official tag composed of a polyurethane body and a metal piercing tip. After disinfecting the tagging site with an alcohol swab, an ear tag was applied to the right ear using an ear tag applicator (Allflex Inc., USA). Subsequently, a 1% povidone-iodine solution was applied to the tagged area. Wounds were assessed at 28 ± 1 days of age and classified according to previously established criteria (Hayer *et al*., 2022; Harmon *et al*., 2023). All procedures in both Stage 1 and Stage 2 were performed by the same experienced operator.

Heart rate measurements: Heart rates were measured using a stethoscope for a 15-second period and extrapolated by four. Measurements were taken immediately after ear-tagging (Minute 0) and subsequently at 15, 60, 180, and 300 minutes after this procedure.

Blood sampling and cortisol analysis: An intravenous catheter (Introcan® I.V. catheter, 16G × 2", Braun Medical Inc., Germany) was placed in the left jugular vein of all calves under aseptic conditions and local anesthesia one day before the experiment. Minute 0 was defined as the time of PPT measurement or ear-tagging. Blood samples were collected immediately after each heart rate measurement. The serum was separated by centrifugation at 1,690 × *g* for 10 minutes and stored at -20°C until analysis. Serum cortisol concentrations were measured using a chemiluminescence immunoassay test kit (ADVIA Centaur® Cortisol assay, SIEMENS, USA) and a chemiluminescence immunoassay analyzer (ADVIA® Centaur XPT Systems, SIEMENS, USA), with a measurement range of 2 to 750 ng/ mL. Additionally, the area under the curve (AUC) of the time-serum cortisol concentration was calculated using the trapezoid method.

Behavior observation: Following ear-tagging, behaviors were recorded and assessed by a trained assistant who was blinded to the treatment groups. Following the methodology of our previous study (Liu *et al*., 2023), two video cameras (DCS-8302LH, D-Link, Taiwan) were installed two meters behind and lateral to the calf pen to record the pain-related behaviors. Videos were recorded for five minutes before each blood sampling, which took place immediately after ear-tagging and at 10, 55, 175, and 295 minutes post-tagging. The recorded pain-related behaviors included head shaking (rapid side-to-side movement of the head), ear flicking (rapid twitching of one or both ears), tail flicking (rapid side-to-side tail wagging), leg movement (kicking or lifting), and back arching (full upward arching of the back) (Faulkner and Weary, 2000; Schnaider *et al*., 2022; Stilwell *et al*., 2010).

Wound score assessment: One week after ear-tagging (at 28 ± 1 days of age), wounds were assessed using a three-point grading scale previously established by Hayer *et al*. (2022). In this system, wound score 1 indicated no visible signs of blood, scabbing, or pus discharge (Figure 3a); score 2 indicated the presence of incrustation or scab with slight bleeding or pus discharge (Figure 3b); and score 3 represented heavy purulent discharge, tissue deformation, or both (Figure 3c). In addition to this scoring system, wound types were further classified according to Harmon *et al*. (2023) into three categories: "Piercing only," referring to wounds without visible tissue changes (Figure 3d); "Piercing crust," characterized by raised, dried tissue at the site (Figure 3e); and "Tissue growth," defined as smooth granulation tissue growing around the piercing site and extending beyond the intact ear surface (Figure 3f). As most wounds exhibited only dry scabs or minor dried exudate, without signs of infection such as severe inflammation, excessive discharge, or fever, no further clinical follow-up was considered necessary.

Statistical analysis: Statistical analyses were conducted using GraphPad Prism Statistics software version 10.4.1 (GraphPad Prism Software Inc., USA). Differences with $P < 0.05$ were considered statistically significant. Values of PPT for the left ear were compared among groups using one-way ANOVA, while differences between the left and right ear within each group were analyzed using a paired t-test. Heart rate, serum cortisol concentration, and behavioral responses were analyzed using a mixed-effects model to compare groups and assess changes across time points within each group. Tukey's HSD test was used for post-hoc comparisons.

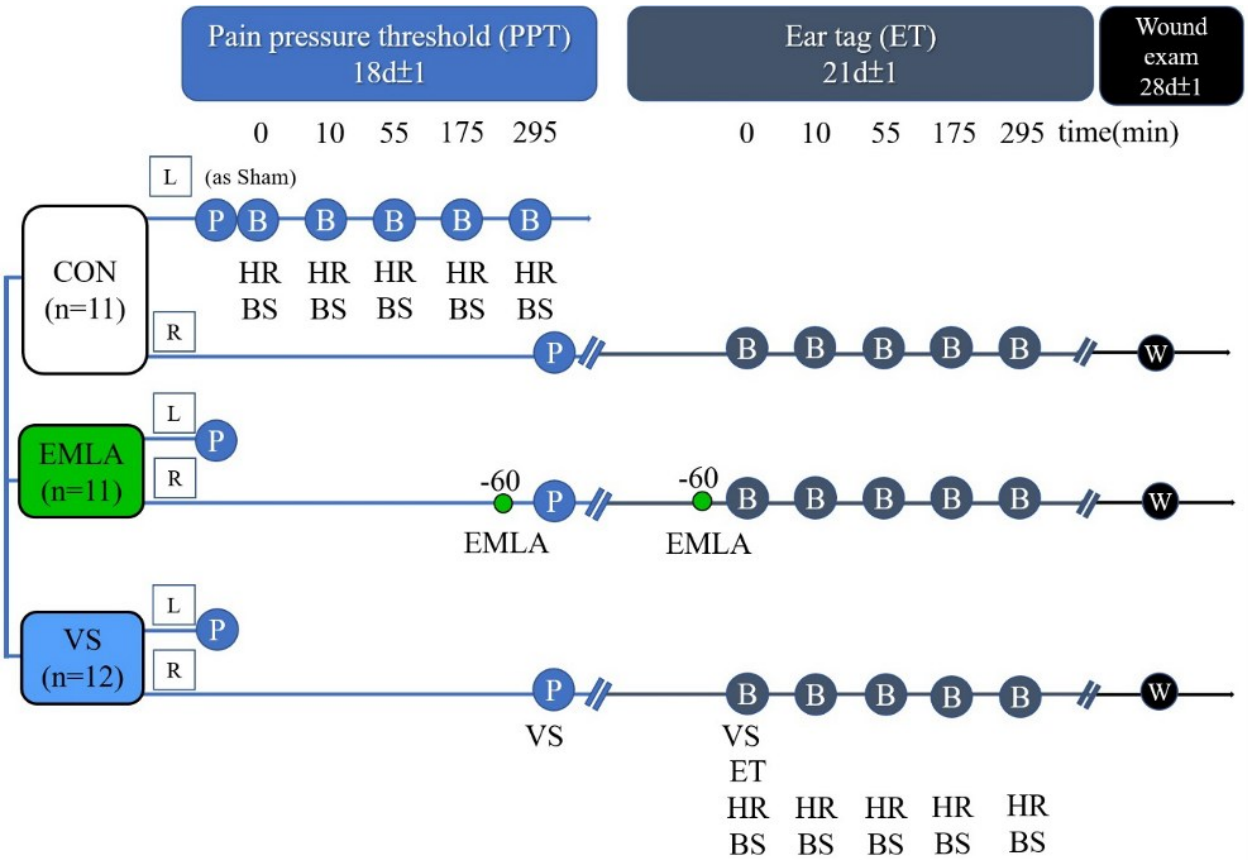

Figure 1 Schematic diagram of the experimental procedure. The time point at 0 minutes was defined as the time when PPT measurement or ear-tagging is completed. B: Behavioral observation recorded over a 5-minute duration; BS: Blood sample collection; CON: Control group; EMLA: Eutectic mixture of local anesthetics group; ET: Ear-tagging. HR: Heart rate measurement; P: Pressure pain threshold measurement; Sham: Sham group; VS: Vapocoolant spray group; W: Wound assessment.

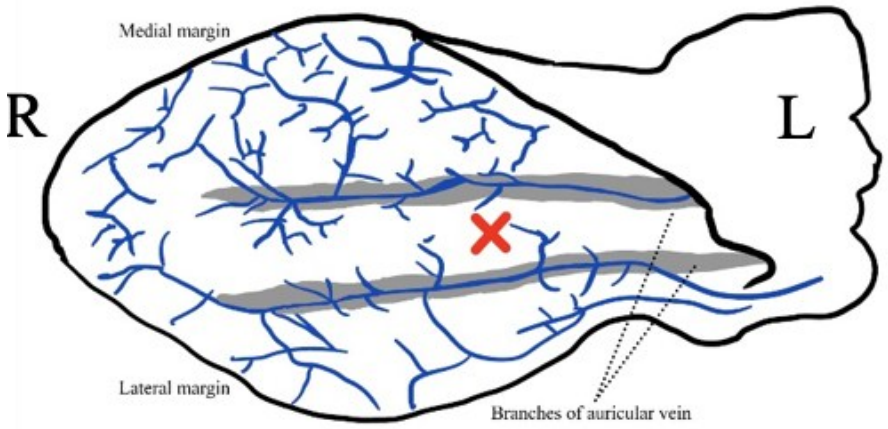

Figure 2 Schematic diagram of the right ear structure in calves. The gray areas represent the two main cartilages of the ear, while the blue lines indicate the branches of the ear vein. The red cross indicates the placement site for ear-tagging. L: left side; R: Right side.

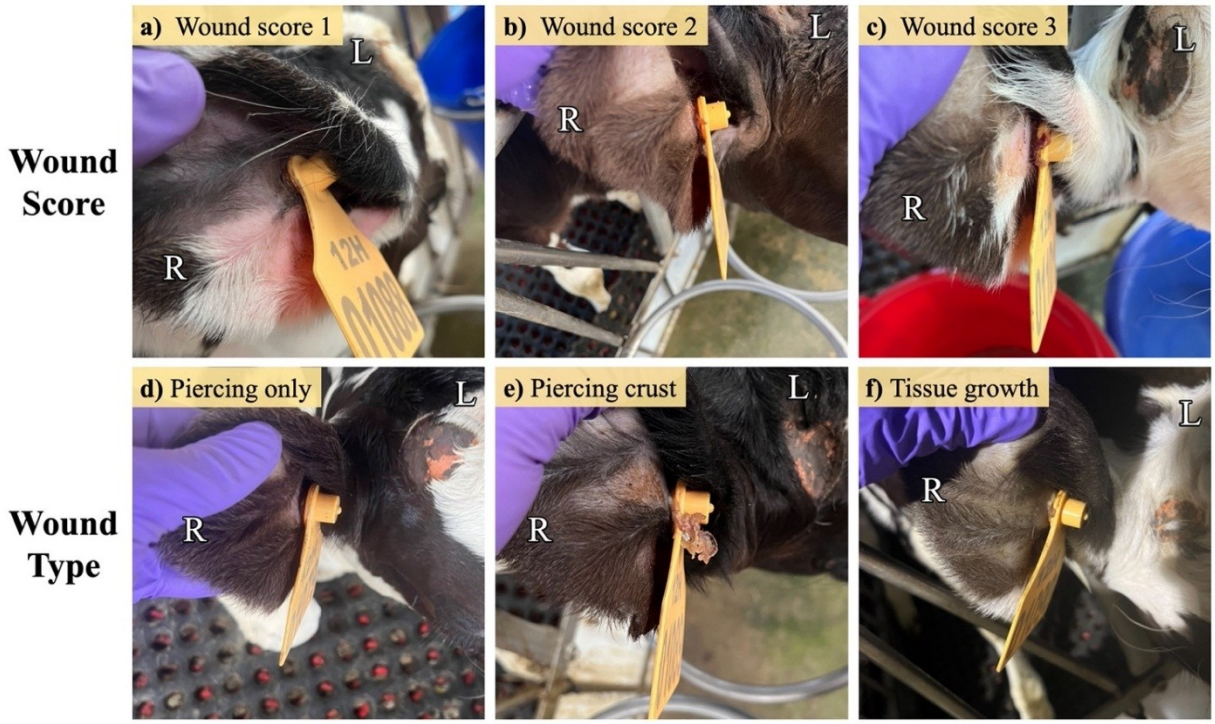

Figure 3 Representative images of ear-tagging wounds classified by severity scores and wound types. a) Wound score 1: no visible signs of blood, scabbing, or pus discharge; b) score 2: the presence of incrustation or scab with slight bleeding or pus discharge; c) score 3: heavy purulent discharge, tissue deformation, or both. Wound types were further categorized as follows: d) Piercing only: no visible wound-related tissue changes; e) Piercing crust: raised, dried tissue at the site; f) Tissue growth: smooth granulation tissue growing around the piercing site and extending beyond the intact ear surface. L: left side; R: Right side.

Result

Pain pressure threshold: In Stage 1, the PPT values for the left ear showed no significant differences in the CON, EMLA, and VS groups (31.7 ± 0.9, 28.9 ± 0.9, and 29.3 ± 0.5 N/cm²). However, the PPT values for the treated right ear were significantly higher in both the EMLA (40.7 ± 1.2 vs. 28.9 ± 0.9 N/cm², $P < 0.0001$) and VS groups (44.4 ± 1.1 vs. 29.3 ± 0.5 N/cm², $P < 0.0001$) compared to their respective left ears. Likewise, the PPT values for the right ear in the EMLA and VS groups (40.7 ± 1.2 and 44.4 ± 1.1 N/cm²) were significantly higher than that in the CON group (31.6 ± 0.8 N/cm², $P < 0.0001$).

Pain-related behaviors after ear-tagging: The total number of head shakes and tail flicks within the first five minutes post-ear-tagging was significantly higher in the CON, EMLA, and VS groups than those at other time points ($P < 0.05$). Similarly, the total number of leg movements was significantly higher within the first 5 minutes post ear-tagging in the CON group ($P < 0.05$), while no significant increase was noted at other time points in the other three groups. However, a comparison of the frequency of ear flicks across time points revealed no significant differences among the Sham, CON, EMLA, and VS groups.

As shown in Figure 4, the highest frequency of pain-related behaviors was predominantly observed in the CON group within the first 5 minutes post ear-tagging. The incidence of head shaking within this period was significantly higher in the CON and VS groups (9.4 ± 1.5 and 9.6 ± 1.9) compared to the Sham and EMLA (2.0 ± 0.6 and 3.0 ± 0.8). Additionally, the frequency of head shaking at 300 minutes post-treatment was significantly higher in the VS group than in the Sham and EMLA groups. The mean frequency of ear flicking was significantly higher in the CON group within the first five minutes post ear-tagging compared to the Sham group (6.0 ± 0.9 vs. 2.2 ± 0.7, $P < 0.01$). At 300 minutes post-tagging, calves in the VS group showed a significantly higher mean value of ear flicking than those in the Sham and EMLA groups.

The frequency of tail flicking within the first 5 minutes post ear-tagging was significantly lower in the Sham and VS groups (20.9 ± 3.0 and 13.1 ± 4.9) compared to the CON group (42.3 ± 5.8), while no significant differences were observed at other time points among the groups. The mean total number of leg movements in the CON group peaked significantly within the first 5 minutes post-ear-tagging (12.9 ± 2.5), compared to the Sham and EMLA groups (2.6 ± 0.9, 2.1 ± 0.4, $P < 0.01$) and VS group (2.8 ± 0.6, $P < 0.05$). However, at subsequent time points, leg movement frequencies remained relatively low and did not differ significantly among the groups. The incidence of back arching was 100% in the calves of the CON and VS groups, while it was observed in only 9.1% of the EMLA group and 0% of the Sham group.

Wound score assessment: At seven days post-ear-tagging, 50%, 54.5%, and 58.3% of calves in the CON, EMLA, and VS groups, respectively, had a wound score of 1. Mild inflammatory signs, including redness, slight swelling, and occasional hemorrhage, were observed in some calves with wound scores of 2 or 3. However, no instances of ulceration, frostbite, allergic

reactions, or other adverse effects were noted following analgesic treatment (Table 1). In addition, 50% of calves in the CON group exhibited minor exudation or scab formation (≥ score 2), compared to 45.5% in the EMLA group and 41.6% in the VS group. Similarly, most ear-tagged calves in the CON, EMLA, and VS groups had wounds classified as piercing without complications (50%, 54.5%, and 58.3%, respectively), while the remaining calves showed signs of tissue repair, such as piercing with crust formation or tissue regrowth.

Cortisol analysis and heart rate: Serum cortisol concentrations were significantly higher in the CON, EMLA, and VS groups, peaking immediately after ear-tagging and remaining elevated at 15 minutes post-PPT, followed by a steady decrease until 300 minutes after (Table 2). Similarly, cortisol levels increased significantly at 0 and 15 minutes after PPT manipulation, then decreased at subsequent time points. However, no significant differences in cortisol levels were noted at any time point among the Sham, CON, EMLA, and VS groups. Likewise, the AUC of the cortisol profiles did not differ significantly among the four groups.

In the CON group, heart rates were significantly higher immediately after ear-tagging compared to those recorded at 180 and 300 minutes post-ear-tagging (113.1 ± 9.3 vs 93.5 ± 4.3 and 94.9 ± 3.5, $P < 0.05$). Similarly, calves in the VS group exhibited a significantly higher heart rate immediately after ear-tagging compared with other time points ($P < 0.05$). However, changes in mean heart rate were not significantly influenced by ear-tagging and showed no significant differences among the groups.

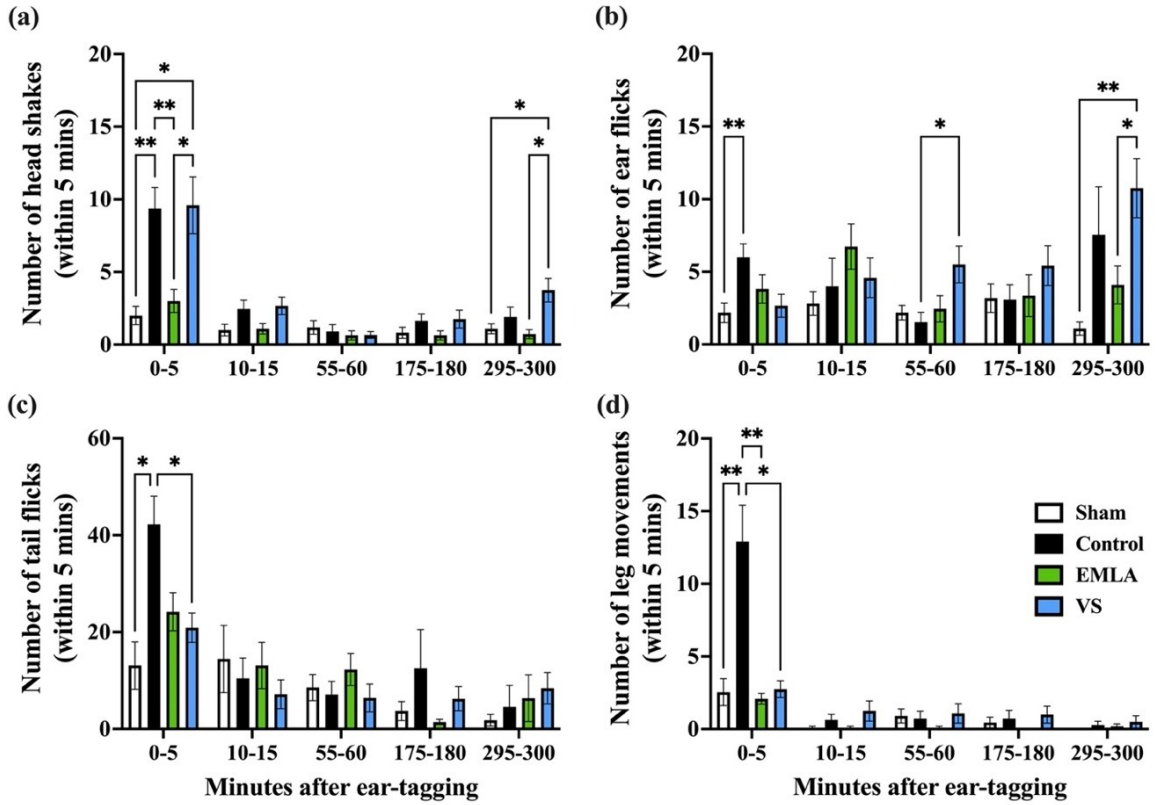

Figure 4 Changes in mean (± SE) frequencies of total pain-related behaviors, including (a) head shaking, (b) ear flicking, (c) tail flicking, and (d) leg movement, in calves at each time point following ear-tagging across treatment groups.

Table 1 Wound scores and types of the calves in the CON, EMLA, and VS groups on day 7 post ear-tagging.

	Group		
	CON (n=10)*	**EMLA (n=11)**	**VS (n=12)**
Wound score			
score 1	5 (50%)	6 (54.5%)	7 (58.3%)
score 2	3 (30%)	2 (18.2%)	4 (33.3%)
score 3	2 (20%)	3 (27.3%)	1 (8.3%)
Wound type			
Piercing only	5 (50%)	6 (54.5%)	7 (58.3%)
Piercing crust	4 (40%)	4 (36.5%)	5 (41.6%)
Tissue growth	1 (10%)	1 (9.0%)	0 (0.0%)

CON: control group; EMLA: eutectic mixture of local anesthetics group; VS: vapocoolant spray group. Wounds were classified into score 1 (no visible signs of blood, scabbing, or pus discharge), score 2 (presence of incrustation or scab with slight bleeding or pus discharge), and score 3 (heavy purulent discharge, tissue deformation, or both). Wound types were categorized as Piercing only (no visible wound-related tissue changes), Piercing crust (raised, dried tissue at the wound site), and Tissue growth (smooth granulation tissue growing around the piercing site and extending beyond the intact ear surface). *One of the calves in CON was removed due to incomplete data on wound assessment.

Table 2 Serum cortisol concentrations (pg/ ml) (mean ± SEM) in the calves of the 4 groups at different time points after ear-tagging or ear-tagging simulation.

Group	n	time relative to ear-tagging				
		after	15 min	60 min	180 min	300 min
Sham	11	9.0 ± 1.4^{ab}	10.1 ± 1.4^{a}	2.7 ± 0.7^{c}	3.4 ± 0.7^{c}	4.6 ± 1.3^{bc}
CON	11	7.1 ± 1.5^{a}	6.6 ± 1.3^{a}	3.6 ± 0.9^{a}	2.5 ± 0.5^{b}	5.0 ± 0.9^{a}
EMLA	11	9.7 ± 2.3^{a}	9.3 ± 1.5^{a}	4.9 ± 1.0^{ac}	2.6 ± 0.4^{b}	4.0 ± 0.7^{bc}
VS	12	6.9 ± 1.0^{a}	5.7 ± 1.1^{a}	2.0 ± 0.0^{b}	4.7 ± 1.0^{a}	5.6 ± 0.9^{a}

$^{a\text{-}c}$Different lower-case superscript letters indicated differences across time within each group. CON: control group; EMLA: eutectic mixture of local anesthetics group; Sham: sham group; VS: vapocoolant spray group.

Discussion

This study primarily evaluated the efficacy of EMLA and VS in mitigating the pain associated with ear tagging. In Stage 1 of the experiment, no significant differences in the PPT values were found among the CON, EMLA, and VS groups in the untreated left ear, with an average pressure tolerance of approximately 28 to 30 N/cm². This finding indicates consistent procedural techniques across groups, minimizing potential bias. During Stage 1, the PPT values of the treated right ear in the EMLA and VS groups were significantly higher than those in the CON group, suggesting that both local anesthetics successfully induced hypoalgesia, thereby increasing the local tissue tolerance to pain. In Stage 2, the frequencies of pain-related behaviors, including head shaking, ear shaking, tail shaking, and leg movement, were significantly higher in the CON group within the first five minutes post-tagging compared with the Sham group, indicating that ear-tagging is a painful procedure. Although pain-related behaviors in the CON group remained elevated at 15 minutes post-tagging compared with the Sham group, the differences were no longer statistically significant, suggesting that ear-tagging may cause short-term distress and discomfort in calves, which was unlikely to persist beyond 15 minutes after the procedure.

When assessing pain-related behaviors after ear-tagging, the frequency of head shaking within the first five minutes, post-tagging, was significantly higher in the CON and VS groups compared to the Sham and EMLA groups. However, no significant differences were observed for other pain-related behaviors compared with the EMLA and Sham groups. These findings suggest that the EMLA group, similar to the Sham group, exhibited significantly reduced pain responses compared to the CON group. Additionally, back arching behavior, which is commonly used to assess lameness disorders in cattle (Hoffman *et al*., 2014), has also been employed to evaluate the anesthetic effects during castration procedures in beef calves (Currah *et al*., 2009). In the present study, back arching was observed in both the CON and VS groups following ear-tagging, whereas this behavior was observed in only 9.09% of calves in the EMLA group. This finding suggested that EMLA offered a superior local anesthetic effect compared with VS. Overall, both EMLA and VS effectively alleviated pain responses following ear-tagging in calves, with EMLA exhibiting superior analgesic effects in local tissues. Furthermore, a follow-up examination at 7 days after ear-tagging revealed no adverse effects, such as frostbite or allergic reactions, confirming the safety of both anesthetic methods within the examined time frame.

Several studies in pediatric medicine have demonstrated that both EMLA and VS effectively alleviate pain-related responses in children during medical procedures. Cohen and Holubkov (1997) reported that applying EMLA 60 minutes prior to an intramuscular injection or administering VS for 15 seconds provided comparable local anesthetic effects. Dalvandi *et al*. (2017) also reported that applying EMLA 45 minutes before catheterization in children aged 6 to 12 years or using a 2-second VS resulted in similar anesthetic efficacy. Currently, these two local anesthetics are widely used for short-term pain relief in various medical procedures, including urgent interventions, intramuscular injections, and catheterizations (Wang *et al*., 2023). In fact, most studies have primarily focused on evaluating the analgesic effects of these treatments shortly after the procedure (Cohen and Holubkov, 1997; Dalvandi *et al*., 2017; Mace, 2017). In the present study, the VS group showed significantly higher frequencies of head shaking and ear flicking 300 minutes after ear-tagging compared to the Sham group. These findings suggested that the anesthetic effect of VS is transient, likely due to its rapid dissipation as tissue temperature returns to baseline through blood perfusion (Lomax *et al*., 2017). In contrast, EMLA demonstrated a longer anesthetic effect, which persisted for at least 300 minutes.

Previous studies have demonstrated the analgesic effects of EMLA and VS in animals. Keating *et al*. (2012) reported that applying EMLA 20 minutes before ear tattooing in rabbits significantly reduced the Rabbit Grimace Scale scores, heart rates, and serum cortisol levels, demonstrating its efficacy in pain management. Similarly, Chung *et al*. (2022) found that applying EMLA 60 minutes before intravenous catheterization in the rabbit ears significantly increased the success rate of the procedure, further supporting its effectiveness as a local anesthetic agent. In addition to EMLA, VS exerts short-term anesthetic effects through localized cooling. Applying VS for five seconds can lower the local temperature by approximately 10°C for up to 16 seconds, resulting in partial inhibition of neural transmission, reduced receptor sensitivity, and transient pain relief. As a result, it significantly reduced the struggling response of calves following ear-tagging or ear-notching procedures (Lomax *et al*., 2017). In the present study, the frequency of head shaking was significantly higher in the VS group than in the EMLA group. This difference could be attributed to auditory stress caused by the spraying sound (Lomax *et al*., 2017; Moon *et al*., 2017). Further research is warranted to better understand this phenomenon.

The results of this study showed that seven days post-tagging, minor exudate or scab formation (wound

score ≤2) occurred in 50%, 45.5%, and 41.6% of calves in the CON, EMLA, and VS groups, respectively. The overall wound recovery rates were higher than those reported in previous studies, which could be attributed to specific management practices implemented in this study. Calves were housed in individual pens with slatted floors raised 35 cm off the ground, and the pens were cleaned twice daily, reducing potential delays in wound healing caused by cross-sucking or environmental contamination. Additionally, calves were fed 3 liters of milk twice daily, which corresponds to approximately 11–12% of their body weight and exceeds the commonly recommended minimum of 10%. This level of nutritional intake was sufficient to support normal growth and may have contributed to the rapid wound healing observed in this study by providing essential energy and nutrients for tissue repair. Previous research has reported varying wound healing rates following ear-tagging. Harmon *et al.* (2023) observed that 85% of calves developed scab formation within seven days post-ear-tagging, whereas Hayer *et al*. (2022) found that approximately 52% of wounds remained unhealed even after 12 weeks post-tagging. It has been suggested that the healing time following ear-tagging is comparable to that required for other routine procedures, such as castration (Norring *et al*., 2017) and disbudding (Adcock and Tucker, 2018). Moreover, several factors influence wound healing, including wound care (Bertone, 1989), nutritional status (Harmon *et al*., 2023), the presence of other penmates (Hayer *et al*., 2022), environmental hygiene, and fly control (Mullens *et al*., 2006). Therefore, this study demonstrated that ear-tagging performed after the application of EMLA or VS did not result in any observable impairment of wound healing within the study period and appeared to be safe under the conditions evaluated.

Several previous studies have indicated that cortisol levels can be used to assess distress and pain responses in animals (Stilwell *et al*., 2009; Winder *et al*., 2018). Although Leslie *et al*. (2010) found that cortisol levels significantly increased following procedures such as ear-tagging, ear-notching, and intra-peritoneal injection in 8-day-old piglets, no significant difference was observed when compared to the sham group. It was speculated that cortisol might not be a reliable indicator of short-term pain due to factors such as human interference or restraint (Leslie *et al*., 2010). In the present study, we also observed that changes in cortisol levels were not effective indicators of distress and pain responses after ear-tagging in calves. This may be attributed to similar confounding factors, which could contribute to the increase in heart rate observed in the sham ear-tagging procedure. Therefore, both cortisol levels and heart rate may not be reliable indicators of distress monitoring following ear-tagging in calves.

Due to the limited number of experimental animals, several limitations should be acknowledged. Although the sham condition—performed on the left ear of the control group during Stage 1 under identical restraint and tactile stimulation—did not involve an independent group and could not assess long-term effects in the absence of ear-tagging, it nonetheless served to demonstrate that handling and tactile procedures alone did not confound the pain-related responses observed after ear-tagging. Additionally, the inclusion of a placebo control in the VS group (e.g., spraying with water) would have helped distinguish the sensory effects of spraying from the pharmacological effects of the vapocoolant. Wound healing was favorable in the present study, and no further follow-up was deemed necessary at 7 days post-tagging; however, additional treatment and monitoring may be warranted beyond this time frame if severe infections are detected. Despite these limitations, both EMLA cream and VS are easy to apply, require minimal skill, and cause little distress during handling. Moreover, both treatments are inexpensive (approximately half a US dollar per application) and fast-acting, making them practical and cost-effective options for on-farm pain mitigation during ear-tagging.

In summary, both VS and EMLA cream significantly increased the PPT following ear-tagging in calves, effectively alleviating pain. These methods are simple to use, cost-effective, and safe, making them suitable for routine use in ear-tagging procedures for improving farm animal welfare. Although EMLA exhibited longer-lasting analgesic effects, the prior application was required. Further studies are needed to determine the optimal timing for EMLA application for calf ear-tagging procedures. When assessing the distress of calves after ear-tagging, pain-related behaviors were more reliable indicators than changes in cortisol levels or heart rate. Behaviors such as head shaking, tail flicking, ear flicking, leg movement, and back arching are easily observable, making them suitable for evaluating pain responses following ear-tagging in calves.

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