

## RESEARCH PAPER

# Topical vapocoolant spray reduces nociceptive response to ear notching in neonatal piglets

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## Abstract

**Objective** To evaluate the efficacy of a vapocoolant spray (VS) to provide local anaesthesia for piglets during ear notching.

**Study design** Randomized study.

**Animals** Eleven 10 day old and forty 3–5 day old Large White piglets.

**Methods** Temperature validation studies using thermocouples and a temperature data logger were conducted on dead and live tissue to determine optimal spray distance and duration to reduce tissue temperature to below 10 °C. A behavioural trial was conducted to assess efficacy for ear notching. Piglets were randomly assigned to one of four treatment groups: positive control (POS;  $n = 10$ ), VS ( $n = 10$ ), lignocaine (LIG;  $n = 10$ ) and negative control (NEG;  $n = 10$ ). Spray was administered to the margin of each ear, from a distance of 10 cm, and for a duration of 2 seconds immediately prior to ear notching. Behavioural observation was used to assess movement and vocal response, with responses categorized as no response (0) and response (1).

**Results** Temperature and tissue validation studies indicated that a 2 second spray from 10 cm reduced tissue temperature to below 10 °C, and reduced response to stimulation of the skin with an 18 gauge needle ( $p < 0.001$ ). There was a significant effect of treatment on response to ear notching ( $p < 0.001$ ). The probability of a piglet to respond to ear notching was 98.7% for NEG

piglets, compared with those treated with VS (5.3%), LIG (1.1%) and sham-notched piglets (0.9%).

**Conclusions and clinical relevance** This study contributes new data on the pain of ear notching in piglets. The application of a topical VS prior to ear notching reduced the antinociceptive response of piglets to the procedure, similar to that of a local anaesthetic. Cryoanaesthesia presents a simple and effective option for reducing the pain response to this simple husbandry procedure.

**Keywords** behaviour, cryoanaesthesia, pain management, piglet, vapocoolant spray.

## Introduction

Piglets undergo several husbandry procedures within the first week of life including ear notching, castration, tail docking and teeth clipping. Ear notching is a common procedure in the Australian pig meat industry, and an important husbandry practice for individual animal and farm management purposes. Ear notching involves cutting one or multiple sections of the edge of the ear into a pattern unique to the property or animal (Leslie et al. 2010). However, these procedures have been found to cause significant pain and distress (Noonan et al. 1994; Leslie et al. 2010); importantly, these are performed in Australia without the use of anaesthesia or analgesia.

The limitations to current pain relief options impede their wide-scale use on-farm. These include time between administration and effect of analgesic

drugs (Anil et al. 2005), high costs associated with multiple injections needed to maintain levels of analgesia and skilled operators or veterinarians to prescribe and administer the anaesthetic or analgesic (de Roest et al. 2009).

Cryoanaesthesia has been investigated in human medicine for the reduction of procedural pain (Mawhorter et al. 2004; Hijazi et al. 2009; Page & Taylor 2010). A reduction in tissue temperature to below 10 °C results in decreased nerve conduction velocity, receptor sensitivity and inhibition of nociception (Denny-Brown et al. 1945; Travell 1955; Kunesch et al. 1987; Algafly & George 2007). Cryoanaesthesia has advantages over other forms of anaesthesia because of its practicality and reduced handling and administration time (Page & Taylor 2010). Topical vapocoolant sprays (VSs) can induce rapid skin anaesthesia through evaporation of the volatile liquid spray from the skin surface (Hijazi et al. 2009; Page & Taylor 2010) and have been shown to significantly reduce immunization and cannulation pain in adults and children (Travell 1955; Cohen Reis & Holubkov 1997; Mawhorter et al. 2004; Farion et al. 2008; Hijazi et al. 2009). A study in children found that a topical vapocoolant was equally effective at reducing immunization pain as a topically applied local anaesthetic cream [Eutectic Mixture of Local Anesthetics (EMLA); Cohen Reis & Holubkov 1997]. Therefore, these sprays potentially offer a rapid, convenient and effective anaesthetic for minor surgical interventions in livestock. The aim of this study was to determine whether a VS, comprising a hydrocarbon propellant in an aerosol canister, could cool tissue to below 10 °C for an adequate length of time to provide local anaesthesia and reduce piglet response to ear notching.

## Materials and methods

### Temperature validation studies

Temperature validation studies in dead and live tissue were conducted to determine the ability of the VS (Animal Ethics Pty Ltd., Australia), comprising a hydrocarbon propellant in an aerosol canister, to reduce tissue temperature to below 10 °C, to measure the duration of the cooling effect and to assess tissue anaesthesia.

#### Dead tissue

Initial temperature validation was conducted in dead tissue to establish optimal spray time and cooling

ability of the VS. For the study, a whole piglet carcass, which had died from natural causes, was sourced from The University of Sydney piggery 'Mayfarm' (Cobbitty, Australia). A standard K-type temperature thermocouple attached to a temperature datalogger (Yu Ching Technology Co. Ltd., Taiwan) was validated against a mercury thermometer using a water bath. The water bath was heated from 3 °C to 40 °C  $\pm$  10 °C and the temperature was maintained using a water bath regulator. The K-type thermocouple was rated for use from  $-200$  °C to  $+1260$  °C with 2.2 °C accuracy according to the manufacturer.

To assess temperature conduction of the ear tissue in response to the VS, the K-type thermocouple was inserted (using a 16 gauge needle) beneath the epidermis of the back of the ear to assess the ability of the VS to cool through the full skin depth. The piglet carcass was placed in a plastic bag (with the thermocouple end attached to the data logger outside of the bag), vacuum sealed using a Cryovac machine (Sunbeam Foodsaver; VIC, Australia) and tied and secured with string. The bag was then placed into the water bath until the ear reached a temperature of approximately 30 °C (estimated live piglet ear temperature) as indicated by the data logger. The carcass was then removed from the water bath and the plastic bag, and the VS was applied to the ear margin from a distance of 10 cm (Fjordbakk & Haga 2011) to the front of the ear at three spray duration times: 1, 2 and 3 seconds. The carcass was returned to the plastic bag, re-vacuumed and re-warmed in the water bath between test spray durations. The data logger recorded the change in temperature of the tissue every 1 second for 1 minute for each spray duration. Data were uploaded to MS Excel (Microsoft, WA, USA) via the Temp Monitor S2 Software (Yu Ching Technology Co. Ltd.).

#### Live tissue

Following successful temperature studies in dead tissue, temperature validation was conducted on live tissue to establish if blood perfusion would affect the cooling ability of the VS. For the study, a 10 day old Large White piglet was removed from its farrowing crate and manually restrained by the animal house manager in ventral recumbency. A 3 cm<sup>2</sup> area of the ear was infiltrated with 1 mL of lignocaine hydrochloride (Lignocaine 20; Troy Ilium, Australia) to anesthetize the tissue. A 16 gauge needle was used as a catheter to insert the thermocouple into the subdermal tissue on the back of the ear. The VS was

applied to the front margin of the ear, from a distance of 10 cm, opposite to the thermocouple insertion site. Spray durations of 1, 2 and 3 seconds were assessed, with the ear allowed to return to normal temperature (approximately 25–30 °C) between each spray. The data logger recorded the change in temperature of the tissue every 1 second for 1 minute for each spray duration. Data were uploaded to MS Excel via the Temp Monitor S2 Software. Following the experiment, the thermocouple was removed, the ear cleaned with iodine and the piglet returned to its farrowing crate.

### Clinical validation of tissue anaesthesia

Nociceptive response was assessed using a superficial skin prick test to determine the ability of the VS to induce tissue anaesthesia. This study was conducted at a commercial piggery using ten 10 day old Large White piglets from the same litter. Piglets were individually removed from the farrowing crate for treatment, for a maximum duration of 5 minutes. To reduce stress, the animal house manager manually restrained piglets in ventral recumbency to allow treatment. Each piglet had a 5 cm<sup>2</sup> area marked with a scorable marker pen on its left and right flanks. These areas were used as test sites to determine anaesthetic efficacy of the spray using the pinprick test. The presence or absence of anaesthesia was determined by recording the response to superficial skin pinprick with an 18 gauge needle (Amarpal et al. 2002; Meyer et al. 2007; Beteg et al. 2011). Each piglet acted as its own control, with each site pinpricked prior to and immediately after spraying with the VS. Local and sensory response was recorded as 'yes' (1) or 'no' (0) reaction, with full body movement, muscle fasciculation or local skin twitch considered as a lack of anaesthesia (1). The VS was administered for 2 seconds (determined as optimal from live tissue studies) to each site from a distance of 10 cm following initial pinprick, and the response recorded. The flank was chosen over the ear as it provided a better surface to assess response to the pinprick test, owing to the firm muscular surface and ease of restraint.

### Effect of a vapocoolant spray on piglet response to ear notching

#### Animals

The study was performed on Large White piglets ( $n = 40$  mixed sex) aged 3–5 days at the University of

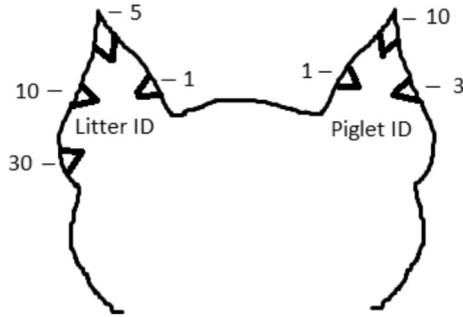
Sydney's Mayfarm piggery. Piglets were raised intensively, and were housed in batches in farrowing crates with their mothers during the trial and for the remainder of the lactation period. Piglets had not undergone any husbandry procedures prior to the trial. Ear notching was performed for identification purposes at days 3–5 according to routine farm practice.

### Study design

The study was conducted in blocks across four litters. For treatment and procedure, piglets were manually restrained by the animal house manager, who also performed the ear notching. Piglets were held gently under the chin with the thumb and forefinger, with the neck and body in the palm of the left hand. Piglets were randomly allocated using a random number generator (GraphPad Software Inc., CA, USA) to one of four treatment groups ( $n = 10$  per treatment), and blocked across litters to ensure equal representation of each treatment within each block.

### Treatments

The positive (POS) control group ( $n = 10$ ; six female and four male animals) involved a sham ear notching procedure, whereby the piglet was restrained, and the ear notching action performed next to the piglets' ears so that they could hear the sound, but no tissue was excised. The negative (NEG) control group ( $n = 10$ ; four female and six male animals) involved performing the ear notching procedure without an anaesthetic intervention, according to industry standards. Piglets in the lignocaine (LIG) group ( $n = 10$ ; six female and four male animals) had 1–1.5 mL of lignocaine hydrochloride (Lignocaine 20; Troy Ilium) infiltrated into the edges of each ear, using a 3 mL syringe and 21 gauge needle, to ensure anaesthesia of the areas to be cut. The VS group ( $n = 10$ ; five female and five male animals) had VS applied to the ear notching sites of each ear (the outer ear margins) prior to notching. The spray was applied for 2 seconds from a distance of 10 cm, based on the optimums established in temperature and tissue validation studies. Each ear was sprayed individually immediately prior to ear notching (i.e. spray left, notch left, spray right, notch right). Spraying did not commence until the piglet was settled (not moving or vocalizing) to improve the objectivity of the behaviour score assigned at the time of procedure. With the piggery manager restraining piglets, this process did not take longer than 1 minute per piglet.



**Figure 1** Ear notching sites for litter and piglet identification (ID). Numbers are added through a combination of notches to match ID.

with an average time from restraint to settle of 23 seconds.

### **Ear notching**

Ear notching was performed by the experienced animal house manager, according to the farm routine procedure. Litter ID was notched with ear notching pliers into the left ear and piglet ID into the right, with the number and combinations of notches varying based on litter and piglet (Fig. 1).

### **Response scoring**

A video camera was mounted on a tripod facing the experimental area to record the piglets' responses. Video was recorded for the duration of the procedure only (approximately 15 seconds per ear, and 30 seconds per piglet), and stopped once the piglet was released from restraint. Only procedural pain response was assessed, as it was expected that there would be no lasting effect of the cryoanaesthesia based on the results of the tissue temperature validation studies (i.e. once tissue temperature increased above 10 °C, it was expected that there would be no anaesthetic effect). Videos were edited prior to scoring to remove the treatment from the videos so that only the procedure and animal response were visible to the observer. Videos were numbered using the identification number of each individual animal. Following the trial, response was scored by a blinded observer using video footage playback on a computer. The observer was shown the videos in random sequence, and scored the piglet reaction to ear notching. Local and sensory response was recorded as 'yes' (1) or 'no' (0) pain response, with vocalization, rapid head withdrawal or body movement considered a pain response to ear notching (1).

### **Statistical analysis**

Because no previous work has been done on this subject, the sample size calculation was based on the following assumptions: the NEG piglets receiving no anaesthesia would have the highest likelihood of response (1) and the LIG and POS piglets would be least likely to respond (0) with an assumed standard deviation (SD) of 1.5. Using the equation  $(a + b) \times 2 \times (SD)^2 / (\text{mean1} - \text{mean2})^2$  and assuming a type 1 error (a) of 5% and a type 2 error (b) of 80%, 23 pain response scores per group would be required. Ten animals per group (three responses per animal, 30 responses per group) were included to allow for error in estimates of the means and SD.

Experimental data from the tissue anaesthesia and ear notching trials were analysed using a general linear mixed model for binomial distribution in GenStat 16th Edition (VSN International, UK). Fixed effects included in the model were treatment, side and litter, with the random effect of piglet. Data are presented as the predicted probability of a response occurring. Wald test *p* values <0.05 were considered significant.

## **Results**

### **Temperature validation studies**

The thermocouple and data logger were found to be accurate to 0.2 °C.

### **Dead tissue**

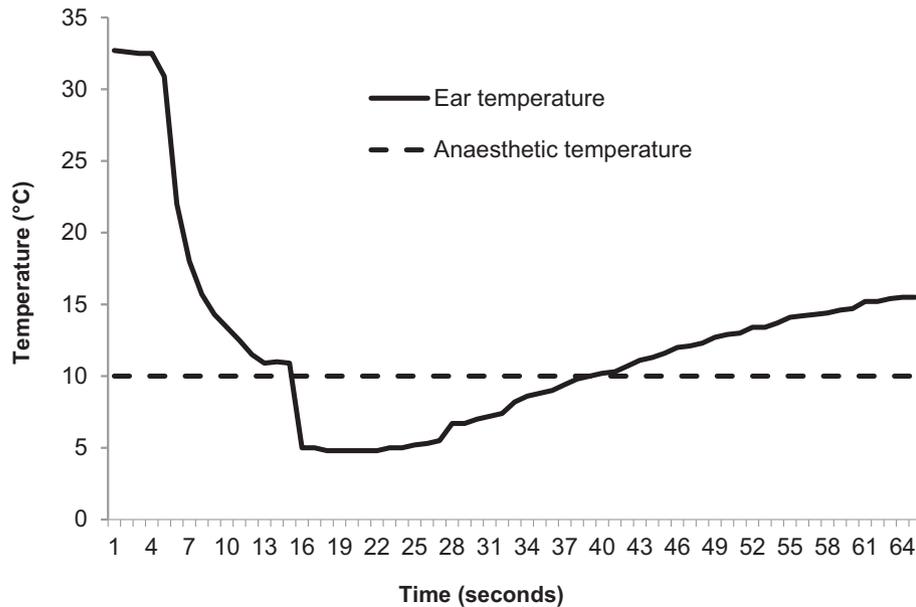
From this study it was determined that a 1 second spray from a 10 cm distance on the ear opposite to where the thermocouple was inserted was adequate to penetrate the ear cartilage and epidermis and reduce the tissue temperature to <10 °C (Fig. 2).

### **Live tissue**

A 2 second spray on the side of the ear opposite to where the thermocouple was inserted was required to penetrate the epidermis and reduce tissue temperature to anaesthetic temperature (<10 °C; Fig. 3). This temperature drop lasted 20 seconds, which would allow sufficient time to conduct the procedure. This methodology was applied to subsequent efficacy studies.

### **Clinical validation of tissue anaesthesia**

There was no significant effect of litter (*p* = 0.136) or side (*p* = 0.751) on piglet response to the pinprick test. The VS significantly reduced nociceptive response to



**Figure 2** Tissue temperature beneath the epidermis of the back of the ear following a 1 second application of the vapocoolant spray from 10 cm on dead tissue. The area beneath the dotted line represents the period at which the tissue was at an adequate temperature to inhibit nociception (<10 °C).

the skin prick test ( $p < 0.001$ ). There was a lower probability that a piglet would respond to a skin pinprick when VS was applied *versus* no VS (Table 1).

#### Effect of a vapocoolant spray on piglet response to ear notching

There was no significant effect of litter ( $p = 0.726$ ) or side ( $p = 1.000$ ) on piglet response; however, there was a significant effect of treatment on response to ear notching ( $p < 0.001$ ). The NEG piglets were more likely to respond to ear notching, compared with the POS, LIG and VS piglets (Table 2). There was no significant difference between responses of POS, LIG and VS piglets (Table 2). There was no significant effect of litter, piglet or side of ear notch on pain response.

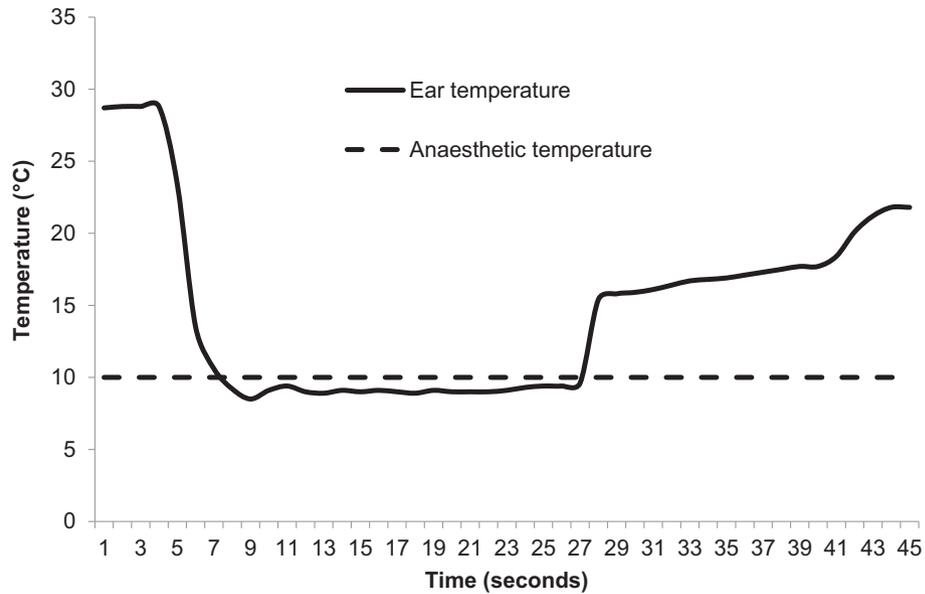
#### Discussion

This study contributes new information on the use of VSs as local anaesthetics on piglet ears. The topical VS significantly reduced tissue temperature, nociception and the behavioural response to ear notching in piglets.

The temperature validation study found that a 2 second spray reduced tissue temperature to below 10 °C for up to 20 seconds. These results align with

similar studies in humans (Travell 1955; Page & Taylor 2010). A study on factors affecting the pain of injection in humans found that a 2 second spray to the forearm with VS was sufficient to cool the skin to below 10 °C (Travell 1955). However, temperature drop in that study lasted only 2–5 seconds, compared with 20 seconds in the current study. The type of tissue targeted likely results in these differences in optimal spray time and rate of tissue cooling. Temperature reduction is less effective at greater tissue depths, particularly greater than 3 cm (Millis 2004). The thin, cartilaginous tissue of piglet ears results in slower tissue reperfusion, and therefore a longer duration of anaesthetic temperature. As ear notching is relatively simple, the duration of anaesthesia achieved in this study (approximately 20 seconds) would allow sufficient time for the operator to perform the procedure. The time taken for the ear temperature to drop below 10 °C was 3 seconds from cessation of spray (Fig. 2). While this is not expected to impact on practicality, this should be assessed in further studies.

Tissue injury can occur when local cooling reaches below  $-20$  °C (Evans et al. 1981). In the current study, tissue temperatures did not reach  $-20$  °C, and there was no observed tissue damage up to 4 weeks postprocedure, indicating that a spray duration of 2 seconds is likely safe and practical for application.



**Figure 3** Tissue temperature beneath the epidermis of the ear following a 2 second application of the vapocoolant spray from 10 cm on live tissue. The area beneath the dotted line represents the period at which the tissue was at an adequate temperature to inhibit nociception (<10 °C).

**Table 1** The probability of a piglet responding (no response = 0; response = 1) to pinprick test with an 18 gauge needle following treatment with a vapocoolant spray (spray) or no treatment (no spray).

Treatment	Probability of response
No spray	0.9572
Spray	0.2075
Wald statistic	21.71
Significance	<0.001

**Table 2** The predicted probability of a piglet responding (0 = no response, 1 = response) to ear notching treatment: POS (no ear notching), VS (vapocoolant spray to ear margin prior to ear notching), LIG (lignocaine injection to ear margin prior to ear notching) and NEG (ear notching only).

Treatment	Probability of response
POS	0.0092
VS	0.0527
LIG	0.0111
NEG	0.9870
<i>p</i>	<0.001
Wald statistic	29.24

The VS significantly reduced the antinociceptive reaction to the pinprick test and ear notching. The pinprick test has been used to determine the presence of anaesthesia in human patients treated with a 2 second spray of a vapocoolant (Travell 1955), and was useful for determining anaesthesia in piglet skin in the current study. Piglets were less likely to respond to the pinprick when the area was treated with the VS, suggesting adequate cooling and blocking of nerve conduction to induce anaesthesia.

The topical VS significantly reduced piglet response to ear notching. These results align with previous published work in calves undergoing ear tagging and ear notching (Lomax et al. 2017). Piglets treated with the VS had responses to ear notching that were comparable with those treated with subcutaneous LIG infiltration. While LIG effectively reduces the procedural pain associated with castration in piglets (White et al. 1995; Prunier et al. 2002, 2006), LIG infiltration is limited for on-farm use by factors including the time between administration and effect (Anil et al. 2005), and cost and requirement of a veterinarian for administration (de Roest et al. 2009). The rapid action and ease of application of the VS make it a practical alternative to other local anaesthesia options. The brief, localized action of the VS will allow farmers to treat the piglet immediately before the procedure, removing the need for double handling or increased time for management

procedures. This is beneficial in terms of time for the farmer as well as reducing the stress to the piglet of prolonged restraint and separation from the mother. Farmers may therefore be more willing to adopt cryoanaesthesia due its practicality and efficacy in reducing the procedural pain. Hydrocarbon sprays are commercially available, however full safety profiles would need to be determined prior to their widespread use in livestock. While the current study indicates efficacy for ear notching, the ability of the VS to induce anaesthesia in other tissues, such as the scrotum or tail of piglets undergoing castration or tail docking, requires further investigation.

We used behavioural observations in the study to assess response to ear notching. Although many pain studies use physiological and endocrine indicators of pain including heart rate and cortisol, these methods can be limited in their indication of procedural pain alone. Endocrine measures such as cortisol concentration may be influenced by other factors such as restraint, stress, physical activity or sexual excitement (Anil et al. 2005; Currah et al. 2009). Observation of acute behavioural response to the procedure allowed us to document procedural pain, with minimal impact from external factors. Observation of antinociceptive reactions to the procedure is a strong indicator of pain, therefore a reduction in these responses is an indication of a reduction of pain. Furthermore, videography allowed playback and editing to remove view and sound of treatment for more accurate behavioural observations (Millman 2013).

The issue of postoperative pain was not addressed in the current study. It is well-documented that husbandry procedures in piglets are associated with behavioural, physiological and endocrine pain responses (Noonan et al. 1994; Prunier et al. 2002; Hay et al. 2003; Moya et al. 2008; Leslie et al. 2010). Ear notching presents a simple model to test efficacy of an option for managing procedural pain. The combined effect with postoperative pain management is warranted for use in more invasive procedures such as castration or tail docking to have a multimodal approach to pain management and to further reduce the welfare impact of these procedures on the animals.

This study contributes new data on the pain of ear notching in piglets. The application of a topical VS prior to ear notching reduced the pain response of piglets to the procedure, similar to that of a local anaesthetic. The investigation of the efficacy of cryoanaesthesia in piglets warrants exploration into

more invasive husbandry procedures such as castration or tail docking, and the combined use with postoperative analgesia.

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## Authors' contributions

SL: design, data acquisition, management and interpretation, preparation of manuscript; EH: data analysis; LO: data collection and statistical analysis, preparation of manuscript; PW: revisions to manuscript, approval of final version.

## Conflict of interest statement

Authors declare no conflict of interest.

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