AUSTRALIAN VETERINARY JOURNAL
AUSTRALIA'S PREMIER VETERINARY SCIENCE TEXT
PRODUCTION ANIMALS

Topical wound anaesthesia: efficacy to mitigate piglet castration pain

ML Sheil,[a]* M Chambers[b] and B Sharpe[b]

Objective There is a critical need for safe and effective analgesic treatments to address pain resulting from surgical husbandry procedures in livestock. Piglet castration results in acute pain and stress to the animal; however, it is performed globally on millions of piglets annually, often without any analgesia what-so-ever. Tri-Solfen® (Animal Ethics Pty Ltd, Yarra Glen, Victoria, Australia) is a combination local anaesthetic and antiseptic formulation which, applied topically to wounds, has proven effective, and is registered for use to alleviate pain associated with castration (and other wounds) in lambs and calves in Australia and New Zealand. It is also reported to be effective to reduce pain in piglets following castration.

Design This randomised, blinded, placebo-controlled study examined the safety and efficacy of the formulation, administered via an adapted wound instillation method, to control pain both during and following piglet castration.

Method Piglets received Tri-Solfen or placebo, instilled to the wound immediately following skin incision. A 30 s wait period was then observed prior to completing castration. Pain mitigation was assessed by grading nociceptive resistance movements and piglet vocal response during castration, as well as by grading response to mechanical sensory stimulation of the wound (von Frey and needlestick) following castration.

Results There was a significant reduction in nociceptive motor and vocal response during castration and in response to mechanical sensory wound stimulation up to and including 2 h following castration. There were no adverse events.

Conclusion Administered via this method, Tri-Solfen is effective to mitigate acute peri-operative castration pain in piglets.

Keywords animal welfare; castration; local anaesthesia; pain management; pigs

Abbreviations AUC, area under the dB/time waveform; NSAID, nonsteroidal anti-inflammatory; NRS, numerical rating scale

Aust Vet J 2020 doi: 10.1111/avj.12930

There is a critical need for safe and effective analgesic treatments to address pain resulting from surgical husbandry procedures in livestock.[1] The challenge is to develop options that are safe, practical and sustainable for on-farm use. In view of the logistics involved, ideally such products (albeit veterinary prescribed) are able to be farmer applied.

*Corresponding author., baysheil@bigpond.net.au
[a]Animal Ethics Pty Ltd, Yarra Glen, Victoria, 3775, Australia; baysheil@bigpond.net.au
[b]Invetus Pty Ltd, Armidale, New South Wales, 2350, Australia

Piglet castration is well documented to result in acute pain and stress to the animal.[2–5] Globally, it is performed on millions of piglets annually, in most cases without any analgesia what-so-ever.[6] Injected local anaesthesia and/or general anaesthesia may be effective to alleviate pain; however, these methods may have welfare impacts in and of themselves (e.g. due to pain of administration, stress of double handling or sedation, and delayed recovery)[7] and/or may be logistically or cost prohibitive due to the requirement for direct veterinary administration. Nonsteroidal anti-inflammatory (NSAID) medications (such as meloxicam) provide some analgesia 2–4 h following the procedure,[8] but do not address the pain of the procedure itself or during the first minutes and hours following the procedure, when pain is acute.[4,7,9–17]

The use of topical wound anaesthesia is a new and evolving field that shows promise as an alternative or adjunctive means of delivering effective pain mitigation to farm animals in these settings.[1] Tri-Solfen® is a combination local anaesthetic and antiseptic formulation which, applied topically to wounds, has proven effective and is registered for use to alleviate pain resulting from castration, and other surgical husbandry wounds, in lambs and calves in Australia and New Zealand.[18–21] It is a viscous liquid formulation containing Lignocaine 5%, Bupivacaine 0.5%, Adrenalin 1: 2000 and Cetrimide 0.5%. It is generally applied topically to coat a wound immediately postcreation. Designed to provide rapid onset wound anaesthesia with prolonged effect, it is applied once-only at the time of the wound creation, following which animals are returned to their dams to recover without further interference.

Specific to castration, the product is applied immediately postskin incision, but immediately prior to clamping and/or cutting the cord and excising the testes. This is to ensure good coating of the remaining cord tissue as well as the cut skin edge, which may both otherwise generate pain in the postoperative period. Administered in this fashion, it has proven effective to reduce pain in piglets up to 4 h following castration.[22] (By contrast, simply spraying lignocaine onto the wound postcastration is reported to be ineffective to mitigate pain.[15])

Sutherland et al[16] reported that 'lying without contact' (considered a behavioural sign of pain in piglets) was reduced in Tri-Solfen-treated piglets, as compared with untreated piglets in the first 180 min following castration. However, there was no evidence of a significant impact on pain during the procedure itself, based on vocal response and cortisol levels. This finding is not unexpected as topical anaesthetics cannot be expected to mitigate procedural pain if applied only immediately prior to the procedure. Nevertheless, the question arises whether, if a longer dwell time is provided, Tri-Solfen may be effective to mitigate the acute procedural pain that occurs when subsequently severing the cords. Applied topically to mucosal tissues, lidocaine has an onset time between 20 and 30 s[23]

suggesting that a relatively short increase in Tri-Solfen dwell time may be effective to anaesthetise the cords. This could be highly beneficial, as applying traction and severing the cords are reported to be the most painful parts of the procedure based on motor and vocal responses.[11,13] This study thus examines whether, in the on-farm setting, topical wound anaesthesia with Tri-Solfen is effective to provide procedural as well as postoperative pain mitigation for piglets undergoing castration if a longer wait time (minimum 30 s) is implemented between applying the dose and severing the spermatic cords.

We hypothesised that topical wound anaesthesia (with Tri-Solfen) applied with a 30 s dwell time would be effective to mitigate pain both during and following subsequent piglet castration. Our aims were to examine evidence of peri-operative pain mitigation.

At present, there is no single validated parameter for measuring pain in piglets. It is generally accepted, however, that piglets react to pain in a number of ways including: physiologically, behaviourally and through resistance movements and vocalisation. An extensive literature review was performed to identify the optimal parameters for assessing pain in piglets during and following castration. This identified:

1 Scoring of nociceptive motor (resistance) movements during the procedure (such as by visual analogue scale or numerical rating scale [NRS]) consistently identifies a marked increase in castrated piglets that may be ameliorated by anaesthetic treatment.[24–27] It is thus considered to provide a sound method of documenting pain and pain alleviation during castration.
2 Analysis of vocal response during the procedure is also a useful parameter for pain assessment, albeit that it is an indirect variable and confounded by piglet vocal response to restraint and handling. Results are therefore less sensitive and robust. The most consistent results are seen when piglets are studied in acoustically separated environments.[13,26–28]
3 Direct wound sensory testing is reported as a sensitive and repeatable measure of postoperative wound pain and pain alleviation with anaesthetic treatments in livestock,[18–21] and the method has been recently validated in piglets postcastration.[22] It is thus considered to provide a sound method of documenting pain and pain alleviation following the procedure.
4 Behavioural analysis is also a well-established variable for documenting postoperative pain; however, postcastration abnormalities in piglet behaviour are subtle and short-lived, and results are inconsistent between trials. The most consistent results are found when specific 'pain-associated behaviours' are conglomerated to give an over-all 'pain-associated behaviour' score, and appear to be greatest in the first 30 min, and up to 3 h following castration.[7–10,12,14–16]
5 Physiological measures (such as an increase in cortisol or Adrenocorticotrophic hormone levels), despite being frequently investigated, do not provide a reliable method of assessing pain or pain alleviation in piglets undergoing castration. These are indirect markers of pain, which result from activation of the Hypothalamic–Pituitary–Adrenal – axis, with cortisol and adrenalin release and activation of the sympathetic nervous system. While useful in some settings, the literature establishes that these are poor indicators of pain associated with piglet castration.[4,24,29–31] These measures are severely confounded by the surgical stress response (a reflex response in which activation of the Hypothalamic–Pituitary–Adrenal axis is triggered by surgical incision or bleeding regardless of the presence or absence of pain). They may be further confounded by the administration of adrenalin which is commonly co-administered with local anaesthetics when used in surgical settings.

Based on these findings, nociceptive motor and vocal responses to castration were chosen as primary and secondary efficacy variables for assessment of pain mitigation during castration, and nociceptive response to wound sensory stimulation was chosen as a primary efficacy variable to assess pain mitigation following castration. A second efficacy variable for pain mitigation following the procedure (postoperative painrelated behaviour) has also been examined (and confirmed) in a large multi-centre complementary field trial which is reported separately. (This was unable to be assessed in the same cohort of piglets undergoing wound sensory testing, as this involves frequent re-handling, and hence disturbance of piglet behaviour.)

Materials and methods

The study was conducted in December 2018 and complied with the following national and international standards: VICH GL9 Good Clinical Practice (issued June 2000); APVMA Data Guidelines – Efficacy and target animal safety general guidelines (Part 8, 01 July 2014), and in compliance with University of New England Animal Ethics Committee approval no. 18-100.

Study design

The study was a placebo-controlled clinical efficacy study, using a randomised blocked design based on body weights measured pretreatment. The experimental unit was the individual animal and the statistical animal was the treatment group. Male piglets (commercial hybrid) were confirmed suitable for enrolment into the study if they were between 3 and 7 days of age, in good health. These were individually identified (uniquely numbered via ear tags), weighed and ranked (heaviest to lightest) and the heaviest and lightest piglets were excluded. The remaining piglets (n = 40, mean weight 2.2 kg, range [1.6–3.0 kg]) were sequentially grouped into blocks of two animals and randomly allocated to Groups 1 and 2 from within each block, such that each of Groups 1 and 2 contained 20 male piglets and had similar group mean and range of body weights, (as assessed and confirmed using Statistix 10.0 [Analytical Software Inc., Tallahassee, FL, USA 2010]). On Day 0 after treatment, animals numbered 20, 26 and 41 were removed from the study due to herniation and subsequently replaced with animals numbered 61, 62 and 17, respectively. This reallocation was statistically assessed confirming that the group mean body weights were similar across treatment groups.

Investigational veterinary product

Tri-Solfen Batch No.:181031–1. Composition: 50 g/L Lignocaine hydrochloride, 5 g/L Bupivacaine hydrochloride, 0.048 g/L Adrenaline (as acid tartrate), 5 g/L Cetrimide.

Placebo product

Blue food dye was added to 0.9% saline solution (Baxter, Batch: W47P5, Expiry: April 2020) at the rate of 2.5 to 250 mL saline

(matching the investigational veterinary product colour and hence indistinguishable postapplication).

Investigational veterinary product and placebo products were administered via a custom designed 1 mL Tri-Solfen applicator with ball-point tip fitted (Prodigy Instruments Pty Ltd, Mount Kuring-Gai, NSW, Aust), which was verified prior to use. Treatment administration (total dose) was: piglets 1–<2 kg – 1 mL; piglets >2–4 kg – 2 mL.

Study animals were housed in farrowing pens (2.1 m × 2.1 m) with their dam and litter mates, and routine management practices were followed. Piglets had constant suckling access to their dam and ad libitum access to potable water. On the day of the study, piglets were removed from their dams and castration and treatment was performed as detailed in the following. Video and sound recording were performed during piglet castration, and wound sensory testing was performed 1 min following the procedure. Following this, piglets were returned to their dams. Clinical observations of animals were performed concurrent with postoperative pain assessments at 1 min, thence 1, 2, 4, 8, 12 and 24 h posttreatment.

Method of castration and treatment:

1. Restrain piglet gently but firmly in Kerbl piglet castration cradle (Albert Kerbl GmbH, Buchbach, Germany);
2. When settled, incise scrotum (including tunica) with one single transverse incision using a sterile scalpel to expose and exteriorise each testis;
3. Immediately apply treatment to wound (40% total dose to each side) to coat the exposed spermatic cords;
4. Wait 30 s;
5. Then, remove testes by severing the cords as per routine procedure;
6. Apply the remaining 20% of the total dose to coat the cut skin edge of the wound.

Video recording during castration

A video-camera recording device (iPhone 8 – Apple Inc., Cupertino, CA, USA) was fixed in position above the cradle, and used to record from time of application of the topical wound treatment to approximately 5 s following the severance of the second spermatic cord (i.e. completion of the castration procedure). Each piglet was clearly identified by placing a number label next to the piglet cradle within the camera view. The behavioural response to castration was assessed off-line, by a blinded assessor, using an NRS intensity scale of 0 to 2 at each of four time points: (1) traction on first testis, (2) cutting first spermatic cord, (3) traction on second testis and (4) cutting second spermatic cord. The nociceptive motor response was graded as: 0 = no motor response, 1 = mild motor response, such as a short-lived leg extension or kicking but no major body resistance movement in the cradle, 2 = marked motor response, such as prolonged leg movements and/or marked body resistance movement in the cradle. Assessment thus documented the handling of each teste (traction) and cutting of each spermatic cord, giving a score (0–2) for each event, and a combined total score (0–8) for the castration procedure.

Audio recording during castration

Sound was recorded with a Zoom H2n Handy Recorder (Zoom North America, Hauppauge, NY, USA), mounted on a stand 50 cm from the snout. Audio recording was time and date stamped to correlate with video data. The recording period commenced 30 s following treatment application to the castration wound, which was marked with a verbal cue, and finished with the removal of the second testicle and settling of the piglet. Sound files were analysed off-line by a sound consultant who was blinded to piglet treatment. For analysis, sound files were downloaded from the Zoom recorder and imported to Pro Tools® (Avid technology Inc., Burlington, MA, USA). They were then placed on the Pro Tools timeline at their time stamp position and synchronised with audio from the video recordings to allow precise identification of cutting points. To allow isolation and comparative quantification of vocal output of piglets during the procedure, the time of commencing traction, and time of cord severance were annotated from the time stamp (correlated with the video data). Screen shots were generated with the same time duration window (x axis) and signal scale (y axis). The Area Under the dB/time (waveform) Curve (AUC) was then calculated (in pixels) using image analysis software (Image-J® U. S. National Institutes of Health, Bethesda, MD, USA), for each of the following periods: (1) from commencing traction until severance of the first spermatic cord, (2) from severance of the first spermatic cord until settled at the end of the procedure, and (3) total recorded procedure (Figure 1).

Wound sensitivity testing

The response of each piglet to sensory testing of the wound site was assessed with a von Frey filament (300 g filament) and thence pinprick (18G, 1.5 inch needle) at 1 min, thence 1, 2, 4, 8, 12 and 24 h posttreatment, by trained staff, blind to piglet treatment. Each assessment was scored on a scale of 0–3, based on Lomax et al[22]: 0 = no motor response; 1 = there was a mild local motor response – including a local muscle twitch, flick of the tail or puckering of the anus; 2 = there was a partial lower body withdrawal response – including lifting the rump from the cradle and/or prominent movements (e.g. extension or escape movements) of the rear legs; 3 = there was a full body withdrawal response – including lifting the rump from the cradle and thoracic movements and/or vigorous movement of both the front and rear legs. Animals were restrained similarly to that used for the castration procedure in the piglet cradle for these assessments. Points of application of the von-Frey or needle tip for each site included: Cut edge; both lateral aspects of castration wound – 2 sites. Intact skin: dorsal and ventral aspects of the castration wound ~3 mm from cut edge – 2 sites. This gave a maximum score of 12 for each test method (von Frey or needle) at each time point.

Statistical methods

Raw data were entered into Microsoft EXCEL 2016 using double-entry techniques. Summary tables were prepared in EXCEL while summary figures were prepared using EXCEL and TIBCO Spotfire S+ 8.2, 2010® (TIBCO software Inc. Palo Alto, CA, USA). Video (nociceptive motor response NRS scores) and Audio AUC data were compared using both parametric t-tests and the equivalent nonparametric test (Wilcoxon rank-sum tests); statistical comparisons were performed using S+®. Mechanical sensory response score data were collated by treatment, location (cut edge and intact skin), time point and method of stimulus (von Frey filaments and 18-gauge needles). Total scores for von Frey, needle-stick and overall were calculated. Scores to 4 h posttreatment

PRODUCTION ANIMALS

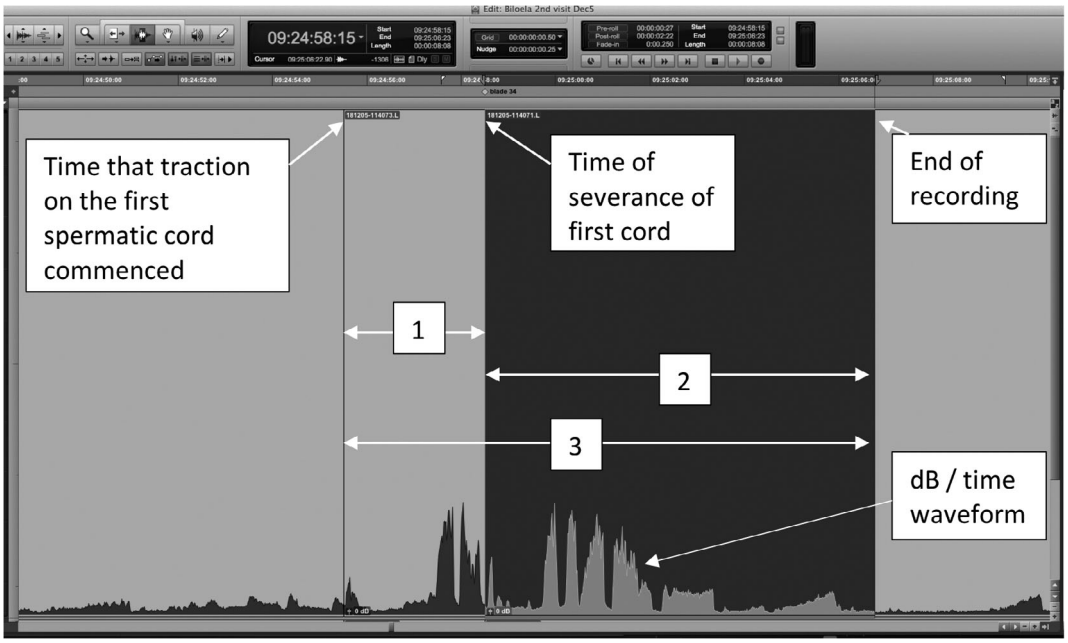

Figure 1. Example screenshot of dB/time waveform output used for image analysis and calculation of 'area under the curve' (AUC) piglet vocal response to castration. AUC was calculated for the following time periods: (1) Commencement of traction on the first spermatic cord until severance of the cord, (2) severance of the first spermatic cord until sound returned to baseline at the end of the procedure, and (3) total of 1 + 2.

Table 1. Group mean nociceptive motor response scores from video assessment (numerical rating scale) of motor response to castration procedures in Tri-Solfen or placebo-treated piglets

Group	Treatment	Traction on first testis (/2)	Cut first spermatic cord (/2)	Traction on 2nd testis (/2)	Cut second spermatic cord (/2)	Total Motor Response score (/8)
1	Placebo	1.5	2.0	1.6	1.8	6.9
2	Tri-Solfen	0.4	1.4	0.4	1.4	3.7

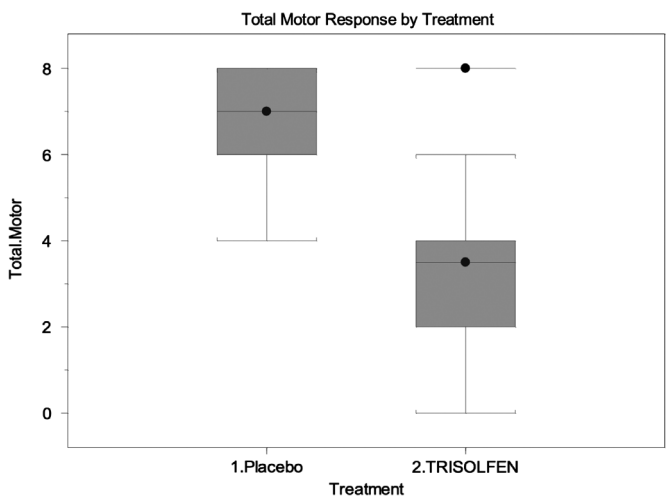

Figure 2. Box plot of total nociceptive motor response to castration, showing a significant reduction ($P < 0.01$) in Tri-Solfen versus placebo-treated piglets.

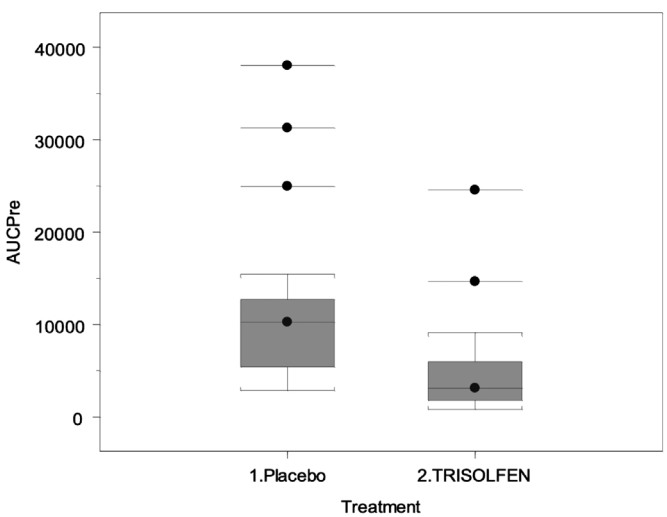

Figure 3. Box plot of vocal response (area under the dB/time waveform or 'AUC') during time period 1 (from commencing traction until severance of the first spermatic cord), showing a significant reduction in vocal response of Tri-Solfen as compared with placebo-treated piglets ($P < 0.01$).

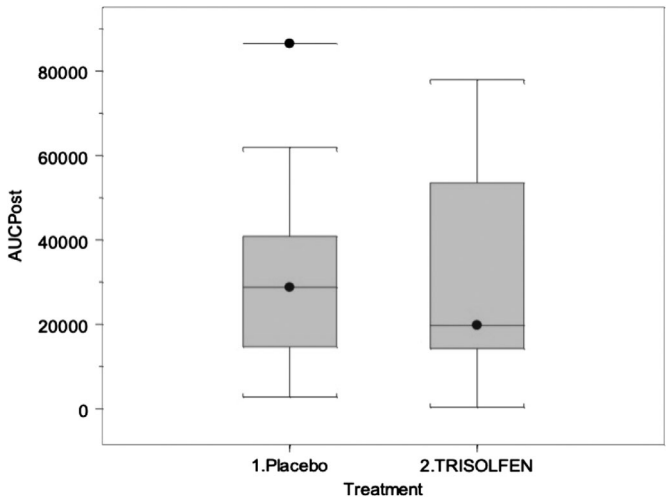

Figure 4. Box plot of vocal response (area under the dB/time waveform or 'AUC') during time period 2 (from severance of the first spermatic cord until the end of the castration procedure). There is wide variability and results are not significantly different between groups.

were compared by treatment, method and over time using Repeated-Measures Analysis of Variance and Statistix 10.0® (2013, Analytical Software, Tallahassee, FL, USA). Suitability of the statistical model was checked via sphericity assumptions, assumptions of covariance and residuals plots. Sphericity assumptions were met and residual plots were generally acceptable although assumptions of covariance were generally not met. Data were therefore also aggregated up to 'response to stimuli/no response to stimuli' based on total score criteria (>2, >3, >4 and >5) and the proportion of piglets responding to stimuli at each time calculated to 8 h posttreatment. Proportions were then compared using Chi-Squared tests and Statistix 10.0.

Results

Video recording during castration

Tri-Solfen-treated piglets demonstrated lower nociceptive motor response scores associated with traction on each teste and cutting of each spermatic cord (Table 1). The total motor response score and the responses to traction on testes (combined) and cutting of spermatic cords (combined) were significantly lower in Tri-Solfen-treated piglets (P = 0.000; P = 0.000; P = 0.004, respectively) than in placebo piglets (Figure 2).

Audio recording during castration

Treatment with Tri-Solfen resulted in a significant reduction in vocal response of piglets to castration as compared with placebo treatment as measured by AUC (mean ± SD of 5070 ± 5667 versus 12,109 ± 9270 pxls, respectively) during the first recorded time period (from commencing traction until severance of the first spermatic cord), (P = 0.007, Figure 3). Numerically lower mean AUC values were recorded in Tri-Solfen-treated piglets, throughout the remainder, or whole of the procedure; however, these differences were not statistically significant, due to wider variability (Figure 4). (A sample size of 20 per group predicted a significant [P < 0.05] reduction in phase 1 AUC with 83% power, versus 10% power for the whole of procedure AUC assessment.)

Wound sensitivity testing

Group mean wound sensitivity scores by test and time point are presented in Table 2 and Figure 5. Placebo-treated animals were more sensitive to both the von Frey filament and needle and at both the cut skin edge and intact skin adjacent to the wound, at 1 min following treatment through to and including 2 h posttreatment. These differences were statistically significant at 1 min and 1 h as shown in Table 3. At 2 h posttreatment, a greater proportion of placebo-treated piglets had wound pain response scores greater than 4 (P = 0.04) or greater than 5 (P = 0.01) than Tri-Solfen-treated piglets. There were no significant differences between groups at 4–24 h following treatment.

Clinical observations and adverse effects

Intestinal herniation (inguinal hernia) occurred in three piglets requiring their removal from the trial as previously noted. One placebo-treated piglet was found dead 24 h posttreatment. Postmortem examination revealed this to be secondary to haemorrhage from the testicular artery stump. There were no other adverse events.

Table 2. Group mean response scores from piglets castrated with Tri-Solfen or placebo treatment to stimulation with von-Frey filament (300 g) or needlestick, by test, site and time point

Site	Test	Group	Treatment	1 min	1 h	2 h	4 h	8 h	12 h	24 h
Cut edge	Von Frey	1	Placebo	0.41	0.48	0.85	1.05	0.85	0.45	1.05
		2	Tri-Solfen	0.05	0.00	0.55	0.85	1.35	0.65	1.10
	Needle	1	Placebo	0.91	1.38	1.35	2.90	2.55	1.70	2.63
		2	Tri-Solfen	0.19	0.30	0.90	2.45	2.95	2.00	2.25
Intact skin	Von Frey	1	Placebo	0.73	0.43	0.50	1.05	0.60	0.60	0.89
		2	Tri-Solfen	0.05	0.10	0.25	1.10	1.20	0.80	0.90
	Needle	1	Placebo	1.64	1.33	1.95	2.60	2.55	1.40	2.42
		2	Tri-Solfen	0.62	0.55	0.95	2.50	2.70	1.90	2.30

PRODUCTION ANIMALS

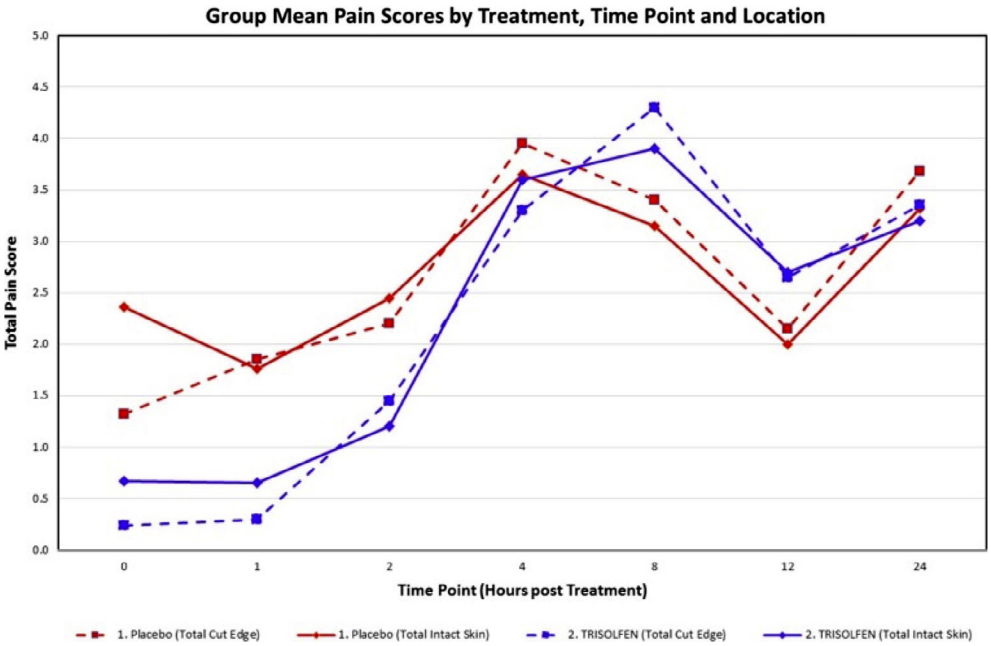

Figure 5. Group mean total wound stimulation response scores (von-Frey filament [300 g] and needle-stick) from piglets castrated with Tri-Solfen or placebo treatment, by site and time point.

Table 3. Statistical analysis of response to von-Frey (300 g) and needle-stick stimulation of the wound following castration in piglets administered Tri-Solfen or placebo during castration

Total all	P-value		
Group	<0.001		
Time	<0.001		
Group × time	0.277		
Sphericity assumptions	√		
Covariance assumptions	*X*		
Group	**Time point**	**Mean**	**Statistical comparison** (values with different letters differ significantly [P < 0.05])
1. PLACEBO	0	3.79	C
2.TRISOLFEN	0	0.85	D
1. PLACEBO	1	3.63	C
2.TRISOLFEN	1	0.95	D
1. PLACEBO	2	4.68	BC
2.TRISOLFEN	2	2.65	CD
1. PLACEBO	4	7.84	A
2.TRISOLFEN	4	6.90	AB

Discussion

The direct application of topical local anaesthesia to wounds, to provide rapid onset wound anaesthesia and postoperative pain mitigation, is a new and evolving field. It is particularly relevant to livestock industries, where there is an acute need for safe and effective analgesic products that may be administered by farmers to address pain associated with surgical husbandry procedures in their animals.

The results obtained in this trial are considered to indicate that in piglets 3–7 days of age undergoing castration in an on-farm setting, topical wound anaesthesia with Tri-Solfen, administered to the wound immediately postskin incision, and followed by a minimum 30 s wait period, effects significant pain mitigation during the subsequent castration procedure, as well as in the minutes and early hours following the procedure.

The application was able to be incorporated into standard castration procedure with only a minimally prolonged handling time of 30 s per piglet. There were no drug-related adverse events reported, consistent with previous reports.[16,22] (The use of two or more cradles, with staggered loading and piglet treatment, would negate any additional handling time for operators).

In this study, we analysed piglet nociceptive motor and vocalisation responses as evidence of castration-related pain and pain mitigation. The literature identifies a reduction in nociceptive motor response as a robust and repeatable method of documenting pain alleviation due to local anaesthesia in piglets. Piglet castration without anaesthesia induces protracted violent struggling and escape behaviour from piglets.[24] This motor response, variously termed 'escape attempts',[32] 'defence behaviour'[26] or 'resistance movements',[27] is usually accompanied by a loud vocal response and is attributable to the nociceptive withdrawal response to acute pain induced during the procedure. Measured using focal assessment, Visual Analogue Scale or NRS, studies have consistently reported that piglet motor response to castration is significantly increased in piglets undergoing castration as compared with sham-handled controls,[32] and/or is significantly ameliorated through the use of general or injected local anaesthetics, indicative of significant pain alleviation.[25–27] Our results, confirming a marked and highly significant reduction of nociceptive motor response in piglets treated with local anaesthetics (as present in Tri-Solfen) applied topically during castration, are consistent with these previous reports, and

indicate that significant procedural pain mitigation is achieved through the use of Tri-Solfen administered via this method.

Piglet vocalisation is also a useful indicator of pain. Although piglets commonly vocalise when they are handled, and particularly when restrained, the literature shows that, during castration, piglets may squeal more often, more loudly and/or at a higher frequency than piglets that are only being held.[9,11–13,26,33] Pulling and severing of the spermatic cords lead to the greatest vocalisation response.[11,13] Piglets castrated without local anaesthesia produce a higher number of screams with higher frequencies compared with piglets castrated with anaesthesia.[13,25–28] A caveat, however, is that in most cases, these findings have been recorded in rooms acoustically isolated from the farrowing pens and using a variety of different methods. In most piggeries, piglet castration is performed in the farrowing pens with rapid through-put to minimise handling times and return piglets to the sow as quickly as possible. Piglet vocal response to each part of the handling and surgical procedures may overlap in this setting, and piglet vocal responses may be impacted by proximity to other piglets and the sow, as well as being confounded by extraneous noise, including squeals of nearby piglets. Our study was designed to assess efficacy 'in-real practice' and was thus performed in the farrowing room. The method of analysis utilised (area under the dB/time waveform measured in pixels) provided a comparable measure of 'total vocal response' from each piglet over the selected time periods. Our result identifying a significant reduction in vocal response during the time from first traction on the spermatic cord until cord severance in Tri-Solfen-treated piglets is consistent with previous reports indicating a reduction in vocal response to castration (and specifically cord traction) in local anaesthetic-treated piglets, considered indicative of significant pain mitigation. While we did not find the same significant difference when comparing vocal responses during the remainder or whole of the procedure, this may reflect wider variability in the duration of the total procedure, and/or piglet response to handling over the longer period, and greater opportunity for confounding.

Direct wound sensory testing was used to assess evidence of pain alleviation following the procedure. Direct sensory testing (such as using von-Frey or needlestick) is a long established and validated method of assessing the efficacy of local anaesthesia and wound analgesia in laboratory research and clinical settings,[34] including in pigs. Castel et al[35] reported the results of studies using von Frey filament assessment of nociceptive threshold to assess incision pain in pigs and impact of local anaesthetic infiltration, and reported that the method was repeatable, and sensitive to the effects of local anaesthetic agents. Modified techniques have been developed for use in livestock species 'in the field'. In this setting, the nociceptive reflex response to needlestick and von Frey Filament application to the wound has been assessed by grading the vigour of the nociceptive withdrawal response at different time points following the procedure. Heightened nociceptive motor response has been documented in the minutes and hours following castration (and other husbandry procedures) in lambs and calves, as compared with sham-handled animals, and/or with preoperative assessments). A significant reduction in nociceptive responses has been documented following Tri-Solfen application to wounds, evident within 1–3 min of application, and persisting for hours following procedures, indicative of significant wound anaesthesia or hypoanaesthesia. This has been associated with reduced postoperative pain-related behaviour in treated animals over the same period.[18–22] Most recently, this method has also been validated as a method of pain assessment in piglets postcastration, including efficacy of local anaesthetic (injected and topical) administration. The authors concluded: 'This method provides a direct measure for wound pain and presence or absence of anaesthesia'.[22] Our results indicating relative hypoanaesthesia in Tri-Solfen-treated piglets as compared with placebo-treated control piglets from 1 min up to and including 2 h following application are consistent with those of Lomax et al[22] and indicative of significant mitigation of wound pain in this period. Lomax et al documented a longer duration of effect (4 h) than was evident in our study (2 h). The dose was the same in both studies; however, there was a slight difference in castration procedure in that Lomax et al used the two (smaller) incision techniques versus the single (larger) incision technique utilised in our study. It is not known if this may have an impact on postoperative wound sensitivity, which may be an area for future study.

Postoperative pain-related behaviour was unable to be assessed in this study, as previously discussed. An additional linked multicentre safety and efficacy trial is reported separately.

We did not include a 'sham' group in this study. Others have demonstrated motor, vocal and wound sensory responses in castrated piglets versus sham animals as documented earlier. This study was designed to investigate amelioration of pain in response to noxious procedures, (cord traction, severing, and so on) which would not be assessable in piglets that did not undergo the procedures. Furthermore, assessments would not be able to be blinded.

Similarly, we have not compared the use of topical wound anaesthesia with injected local anaesthesia. Others have demonstrated motor, vocal and wound sensory responses in piglets castrated with and without injected local anaesthesia as documented earlier. The intent of this study was to investigate topical wound anaesthesia as a stand-alone treatment (such as for use in situations where injected anaesthesia is not available). As both methods of administration result in a significant reduction in vocal and motor responses, it may be concluded that large numbers would be required to detect a significant difference between the two treatments for these variables. While this may be of interest, ultimately, the greatest point of difference between injected local anaesthesia and topical wound anaesthesia, (such as via application of Tri-Solfen) stems from the disadvantages of each method of administration. Injected local anaesthesia provides preoperative skin anaesthesia; however, it requires veterinary presence, induces pain of injection and may pose risks due to inadvertent intravascular administration. It also requires a 3–15 min wait time resulting in the need for, and stress of, double handling and separation from the sow. Topical wound anaesthesia avoids the need for painful injection, double handling and veterinary presence; however, it does not provide anaesthesia for skin incision. Nevertheless, skin incision (performed in a matter of milli-seconds), is considered the least painful part of the procedure, and, with topical wound anaesthesia effective within 30 s, pain is very short-lived. True comparator studies would need to examine the impact of these differences in total, (preprocedure and postprocedure) to gain a true understanding of comparative piglet welfare. Again, this is considered an area for future study.

Additionally, there is the potential for topical wound anaesthesia to be used as part of a multimodal approach to pain mitigation in piglets. Synergistic analgesic efficacy has been reported when Tri-Solfen is combined with NSAID medications in other species,[36] and topical wound anaesthesia may provide enhanced postoperative analgesia when used in combination with NSAIDs or with general anaesthesia. These are also areas for future study.

It is concluded that in piglets 3–7 days of age undergoing castration in an on-farm setting, topical wound anaesthesia with Tri-Solfen, administered immediately postskin incision followed by a minimum 30 s wait period, effects highly significant pain mitigation during the subsequent castration procedure, as well as in the minutes and early hours following the procedure. Significant pain control is thus achieved during the time-periods associated with maximum pain in piglets undergoing this procedure.

Acknowledgments

The authors thank Leonora Pearson, DipRQA (Quality Assurance) Invetus Pty Ltd, Armidale, NSW 2350, Timothy Pearce, Cert. Audio Eng. (Sound Analysis), Annandale, Sydney 2038 and BettaPork Pty Ltd, Valentine Plains Queensland, Australia.

Conflicts of interest and sources of funding

Research was sponsored by Animal Ethics Pty Ltd, and carried out by independent veterinary research company Invetus Pty Ltd, to GCP and VICH standards, to address national and international veterinary medicines regulatory approval requirements. Dr Sheil is an inventor of Tri-Solfen®, a Founding Director and indirect Shareholder of Animal Ethics Pty Ltd.

References

1. Windsor PA, Lomax S, White P Progress in pain management to improve small ruminant farm welfare. *Small Ruminant Res* 2016;142:55–57.
2. Prunier A, Bonneau M, Von Borell EH et al. A review of the welfare consequences of surgical castration in piglets and the evaluation of non-surgical methods. *Anim Welfare* 2006;15:277.
3. von Borell E, Baumgartner J, Giersing M et al. Animal welfare implications of surgical castration and its alternatives in pigs. *Animal* 2009;3:1488–1496.
4. Rault J-L, Lay DC, Marchant-Forde JN Castration induced pain in pigs and other livestock. *Appl Anim Behav Sci* 2011;135:214–225.
5. Leaders MA, Calculator VS, Network ACA. Welfare implications of swine castration. Backgrounder, 2013. Available at: https://www.avma.org/KB/Resources/LiteratureReviews/Pages/Welfare-Implications-of-Swine-Castration.aspx?mode=mobile&PF=1 Accessed 3 May 2017].
6. Fredriksen B, Font I, Furnols M et al. Practice on castration of piglets in Europe. *Animal* 2009;3:1480–1487.
7. McGlone JJ, Hellman JM Local and general anesthetic effects on behavior and performance of two- and seven-week-old castrated and uncastrated piglets. *J Anim Sci* 1988;66:3049–3058.
8. Keita A, Pagot E, Prunier A et al. Pre–emptive meloxicam for postoperative analgesia in piglets undergoing surgical castration. *Vet Anaesth Analg* 2010;37:367–374.
9. Wemelsfelder F, van Putten G. Behaviour as a possible indicator for pain in piglets. Report Research Institute for Animal Production "Schoonoord" (Netherlands), 1985. Available at: http://agris.fao.org/agris-search/search.do?recordID=NL8582592 Accessed 4 June 2019.
10. McGlone JJ, Nicholson RI, Hellman JM et al. The development of pain in young pigs associated with castration and attempts to prevent castration-induced behavioral changes. *J Anim Sci* 1993;71:1441–1446.
11. Taylor AA, Weary DM Vocal responses of piglets to castration: identifying procedural sources of pain. *Appl Anim Behav Sci* 2000;70:17–26.
12. Taylor AA, Weary DM, Lessard M et al. Behavioural responses of piglets to castration: the effect of piglet age. *Appl Anim Behav Sci* 2001;73:35–43.
13. Marx G, Horn T, Thielebein J et al. Analysis of pain-related vocalization in young pigs. *J Sound Vib* 2003;266:687–698.
14. Hay M, Vulin A, Génin S et al. Assessment of pain induced by castration in piglets: behavioral and physiological responses over the subsequent 5 days. *Appl Anim Behav Sci* 2003;82:201–218.
15. Burkemper MC, Pairis-Garcia MD, Moraes LE et al. Effects of oral meloxicam and topical lidocaine on pain associated behaviors of piglets undergoing surgical castration. *J Appl Anim Welf Sci* 2019:1–10. https://doi.org/10.1080/10888705.2019.1590717.
16. Sutherland MA, Davis BL, Brooks TA et al. Physiology and behavior of pigs before and after castration: effects of two topical anesthetics. *Animal* 2010;4:2071–2079.
17. Gottardo F, Scollo A, Contiero B et al. Pain alleviation during castration of piglets: a comparative study of different farm options. *J Anim Sci* 2016;94:5077–5088.
18. Lomax S, Sheil M, Windsor PA Impact of topical anaesthesia on pain alleviation and wound healing in lambs after mulesing. *Aust Vet J* 2008;86:159–168.
19. Lomax S, Dickson H, Sheil M et al. Topical anaesthesia alleviates short-term pain of castration and tail docking in lambs. *Aust Vet J* 2010;88:67–74.
20. Lomax S, Windsor PA Topical anesthesia mitigates the pain of castration in beef calves. *J Anim Sci* 2013;91:4945–4952.
21. Cuttance E, Mason W, Yang D et al. Effects of a topically applied anaesthetic on the behaviour, pain sensitivity and weight gain of dairy calves following thermocautery disbudding with a local anaesthetic. *N Z Vet J* 2019;67:295–305.
22. Lomax S, Harris C, Windsor PA et al. Topical anaesthesia reduces sensitivity of castration wounds in neonatal piglets. *PLoS One* 2017;12:e0187988.
23. Adriani J, Zepernick R, Arens J et al. The comparative potency and effectiveness of topical anesthetics in man. *Clin Pharmacol Ther* 1964;5:49–62.
24. Walker B, Jäggin N, Doherr M et al. Inhalation anaesthesia for castration of newborn piglets: experiences with isoflurane and isoflurane/NO. *J Vet Med A Physiol Pathol Clin Med* 2004;51:150–154.
25. Horn T, Marx G, von Borell E Behavior of piglets during castration with and without local anesthesia. *DTW Dtsch Tierarztl Wochenschr* 1999;106:271–274.
26. Leidig MS, Hertrampf B, Failing K et al. Pain and discomfort in male piglets during surgical castration with and without local anaesthesia as determined by vocalisation and defence behaviour. *Appl Anim Behav Sci* 2009;116:174–178.
27. Hansson M, Lundeheim N, Nyman G et al. Effect of local anaesthesia and/or analgesia on pain responses induced by piglet castration. *Acta Vet Scand* 2011;53:34.
28. White RG, DeShazer JA, Tressler CJ et al. Vocalization and physiological response of pigs during castration with or without a local anesthetic. *J Anim Sci* 1995;73:381–386.
29. Kluivers-Poodt M, Hopster H, Spoolder HAM. *Castration under anaesthesia and/or analgesia in commercial pig production*. Lelystad, Netherlands, Report Animal Sciences Group van Wageningen, 2007;92. http://www.asg.wur.nl.
30. Bonastre C, Mitjana O, Tejedor MT et al. Acute physiological responses to castration-related pain in piglets: the effect of two local anesthetics with or without meloxicam. *Animal* 2016;10:1474–1481.
31. Pérez-Pedraza E, Mota-Rojas D, Ramírez-Necoechea R et al. Effect of the number of incisions and use of local anesthesia on the physiological indicators of surgically-castrated piglets. *Int J Vet Sci Med* 2018;6:159–164.
32. Marchant-Forde JN, Lay DC, McMunn KA et al. Postnatal piglet husbandry practices and well-being: the effects of alternative techniques delivered separately. *J Anim Sci* 2009;87:1479–1492.
33. Weary DM, Braithwaite LA, Fraser D Vocal response to pain in piglets. *Appl Anim Behav Sci* 1998;56:161–172.
34. Curatolo M, Petersen-Felix S, Arendt-Nielsen L Sensory assessment of regional analgesia in humans. A review of methods and applications. *Anesthesiology* 2000;93:1517–1530.
35. Castel D, Sabbag I, Meilin S The effect of local/topical analgesics on incisional pain in a pig model. *J Pain Res* 2017;10:2169–2175.
36. Paull D, Lee C, Colditz I et al. The effect of a topical anaesthetic formulation, systemic flunixin and carprofen, singly or in combination, on cortisol and behavioural responses of Merino lambs to mulesing. *Aust Vet J* 2007;85:98–106.

(Accepted for publication 28 January 2020)