Assessment and amelioration of pain for improved welfare during routine husbandry procedures in piglets

Final Report
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Executive Summary

This application was developed as an extension of our current work on pain assessment and amelioration for improved welfare in grazing livestock. Our proposal will address the APL Core Objective "Genetics, Reproduction and Welfare", specifically as improving welfare associated with routine husbandry procedures in piglets will meet the Key Priority area: Intervention Strategies for Key Management Practices to Maximise Welfare Outcomes.

Routine husbandry procedures for management of piglets include tail-docking, teeth clipping, ear notching and castration. These procedures are widely recognised as painful although detailed work on assessment of pain and the welfare concerns that they incur is yet to be extensively documented in piglets. Furthermore, practical and affordable options for the amelioration of procedural and post-procedural pain are largely undeveloped and unavailable in both the Australian and international pig industries.

Currently our work has broadened to include pilot studies on pre-surgical analgesia and anaesthesia options, and the extension of our studies into topical and cryo-anaesthesia for piglet husbandry procedures. Cryo-anaesthesia is an attractive option for animal husbandry procedures because it is non-invasive and potentially practical, safe, quick and economical to administer. Nerve conduction slows as skin temperature falls and skin becomes anaesthetised at a temperature between 3 and 10 degrees. Preliminary studies reported here have confirmed that skin temperature can be rapidly lowered to anaesthetic levels using this technique. Live animal studies in piglets have determined the optimum spray times for performing each of the husbandry procedures, as well as confirm the extent of pain alleviation that can be achieved.

We have reported on the efficacy of a topical anaesthetic formulation (Tri-Solfen, Bayer Animal Health, Australia) for pain alleviation of castration wounds in piglets. Further work is needed to determine combined efficacy with other commercially available analgesics, and with perioperative treatment with vapocoolant spray.

This research shows great promise for pain management of piglet husbandry. Future work looks to expand our studies to produce practical and affordable combined pain alleviation for tail-docking, teeth clipping, ear notching and castration of piglets. This work has the potential to promote the Australian pig industry as a leader in the provision of animal welfare solutions.

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1. Background to Research

Piglets undergo several husbandry procedures within the first week of life including ear notching, castration, tail docking and teeth clipping. These procedures are widely recognised as painful although detailed work on assessment of pain and the welfare concerns that they incur is yet to be extensively documented in piglets. Furthermore, practical and affordable options for the amelioration of procedural and post-procedural pain are largely undeveloped and unavailable in both the Australian and international pig industries.

Over recent years, the shift in public attitudes around the infliction of pain on animals has led to growing objection to procedures, especially in neonates, that cause pain, even when they are justified for animal health or production management reasons. Consumer and animal advocacy group activism is increasing the pressure on retailers to boycott industry products. Governments are being lobbied to legislate against these procedures when performed without effective pain management. In the European Union for example, many countries have already passed legislation banning castration of piglets unless analgesia and/or anaesthesia is used. In other countries, supermarket chains are refusing to take products from pigs unless they were castrated with analgesia. These developments reflect the growing demand for humane management of livestock throughout the world, and set a challenge to better understand pain as an welfare issue and to develop effective and practical methods of pain alleviation that are applicable to intensive farming operations as in pig production.

Limitations to current pain relief options impede 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). Huxley and Whay (2007) reported that for the majority of producers, the cost of analgesic agents was a significant issue deterring them from adopting such practices.

Tri-Solfen (Bayer Australia) is a commercially available topical anesthetic (TA), hemostatic and antiseptic agent for the alleviation of mulesing pain in sheep. It consists of lignocaine (40.6 g/L), bupivacaine (4.5 g/L), adrenalin (24.8 mg/L) and cetrimide (5.0 g/L) in a gel base. It has been reported that Tri-Solfen is effective in alleviating wound pain of mulesing, castration and tail docking in sheep, and improves wound healing. (Lomax *et al.*, 2008, Lomax *et al.*, 2010, Lomax and Windsor, 2013, Lomax *et al.*, 2013). Due to the efficacy of this TA for reducing surgical pain in lambs, and the similar anatomical nature of the wounds, we hypothesized that a comparable effect would be seen in piglets. Thus this research examines the efficacy of TA for reducing the pain of castration wounds in piglets.
Currently our work has broadened to include pilot studies on pre-surgical analgesia and anaesthesia options, and the extension of our studies into topical and cryo-anaesthesia for piglet husbandry procedures. Cryoanaesthesia has been investigated in human medicine for the reduction of procedural pain (Mawhorter *et al.*, 2004, Hijazi *et al.*, 2009, Page and 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 and George, 2007). Cryoanaesthesia has advantages over other forms of anaesthesia due to its practicality and reduced handling and administration time (Page and Taylor, 2010). Topical vapocoolant sprays can induce rapid skin anaesthesia through evaporation of the volatile liquid spray from the skin surface (Hijazi *et al.*, 2009, Page and Taylor, 2010). Topical vapocoolant sprays therefore offer a potentially quick, convenient and effective anaesthetic for minor surgical interventions in livestock.

Prototype vapocoolant sprays have been designed (AE Pty ltd and Bayer Animal Health Australia), and preliminary studies (using animal skins) have confirmed that skin temperature can be rapidly lowered to anaesthetic levels using this technique. Live animal studies in piglets will be conducted to determine the optimum skin temperature for performing each of the husbandry procedures, as well as to confirm the extent of pain alleviation that can be achieved and monitor the impact on recovery and wound healing. If used in combination with post-operative topical anaesthesia (Tri-Solfen®), extended pain alleviation throughout the procedure and post-operative period may be achieved

The Australian pig industry has correctly identified that welfare research is urgently needed to ensure the corporate image of fresh pork remains strong. Our approach offers APL an opportunity to launch 'Australian pork' as a preferred commodity for meat consumers as it is adopting best practice welfare solutions to improving husbandry procedures. In a very competitive global market for pig products, provision of an effective, practical and affordable solution to pain management in husbandry through producer-applied topical anaesthesia and pre-surgical anaesthesia options, is both a potential marketing edge in the short term, and a probable necessity in the long term, for building consumer demand.

This research shows great promise for pain management of piglet husbandry. We are now seeking to expand our studies to produce practical and affordable solutions for pain assessment and management during tail-docking, teeth clipping, ear notching and castration of piglets. This work has the potential to promote the Australian pig industry as a leader in the provision of animal welfare solutions.

2. Objectives of the Research Project

It should be noted that extent of original application was intended for a fully funded project. Funding received was for pilot studies in the area so objectives were minimized to the below:

1	Develop protocols for assessment of pain of neonatal pigs during castration, tail docking, ear-notching
2	Determine the efficacy of pre-operative cryo-anaesthesia for reducing procedural pain during castration, tail-docking, and ear-notching
3	Determine the efficacy of cryo- and/or topical anaesthesia for amelioration of post-operative pain of castration, tail-docking, ear-notching and teeth clipping

3. Introductory Technical Information

Castration, tail-docking, teeth-clipping and ear-notching are routine husbandry procedures performed within the first few days of life in commercial piggeries around the world. Extensive research has been conducted to assess the effect of these procedures on the welfare of the piglets involved, and it has been widely proven that husbandry procedures cause significant stress, pain and discomfort that can persist for up to 4 days (McGlone *et al.*, 1993, Noonan *et al.*, 1994, Taylor and Weary, 2000, Hay *et al.*, 2003, Gallois *et al.*, 2005, Prunier *et al.*, 2005, Moya *et al.*, 2008, Sutherland *et al.*, 2008).

A vast amount of research has utilized behavioural observation as a technique for assessing pain associated with castration, tail-docking, teeth-clipping and ear-notching in piglets (McGlone *et al.*, 1993, Noonan *et al.*, 1994, Hunter *et al.*, 2001, Hay *et al.*, 2003, Sutherland *et al.*, 2008), with disruption to or increases in stereotypic behaviours being indicative of significant pain response.

Physiological measures, specifically cortisol, are commonly used to assess pain and stress in all species of livestock including piglets (Gonzalez *et al.*, Fox *et al.*, 1994, Lester *et al.*, 1996, Dinniss *et al.*, 1997, Prunier *et al.*, 2005, Moya *et al.*, 2008), with significant rises associated with surgical interventions being attributed to a rise in stress and pain response.

Sutherland *et al* (2008) studied the acute physiological and behavioural responses to tail docking in young piglets (6 days of age) and they found significant rises in cortisol and disruption to behaviour were attributed to tail-docking as compared to un-docked controls. We will be adopting a similar approach to the methodologies of this paper, utilizing behavioural observations and ethogram, and measuring cortisol to firstly assess the pain response of the piglets to castration, tail-docking, teeth-clipping and ear-notching, and further applying these techniques to assess the efficacy of analgesic intervention.

Despite the pain associated with these procedures, they are routinely performed without anaesthesia or analgesia in Australia and many other nations due to the fact that many anaesthetic techniques which are used routinely for comparable surgeries in human and veterinary medicine (such as general anaesthesia or sedation, local anaesthetic infiltration and / or local or regional nerve blockade) are either too complex, costly, time consuming or traumatic to be practical and / or affordable for use in this setting. There have been a number of publications regarding the use of analgesic/anaesthetic interventions to address the pain associated with piglet husbandry procedures (McGlone and Hellman, 1988, McGlone *et al.*, 1993, White *et al.*, 1995, Prunier *et al.*, 2002, Rand *et al.*, 2002, Jaggin *et al.*, 2006) however it is widely recognized that while the options provided (lidocaine injection, general anaesthesia) are effective at reduced pain responses, they are neither practical nor affordable options.

In the European Union, many countries have legislated to ensure the incorporation of local anaesthesia prior to surgical castration, and there have been various cost analyses in response to this (Jaggin *et al.*, 2006, Kluivers-Poodt *et al.*, 2007, Roest *et al.*, 2009) which have found that these methods (general and local anaesthesia) are costly in terms of labour and cost of product. Both Roest (2009) and Kluivers-Poodt (2007) found that the use of analgesia during the castration of piglets significantly increases the labour requirement on farms and, depending on the treatment, the labour of the veterinarians (veterinarians are required to administer S4 restricted drugs including lidocaine). There are also the additional costs for the medications required (anaesthetic and analgesic).

The use of cold temperatures for local anaesthesia and pain relief has been around for thousands of years. Cryoanaesthesia using a vapocoolant spray has been shown to provide an effective local anesthetic prior to intravenous cannulation in children (Farion, Splinter, Newhook, Gaboury, & Splinter, 2008) and adults (Hijazi, Taylor, & Richardson, 2009; Page & Taylor, 2010). Other studies have demonstrated effective skin anaesthesia from a topical vapocoolant spray prior to immunization injections (Mawhorter et al., 2004; Reis, Jacobson, Tarbell, & Weniger, 1998) and minor dermatological procedures such as botulinum toxin injections (Engel, Afifi, & Zins, 2010; Weiss & Lavin, 2009). Numerous trials have shown effective use of cryotherapy in the management of pain associated with sports injuries (Swenson, Swärd, & Karlsson, 1996).

When using a vapocoolant spray, reduction in tissue temperature is achieved via evaporative cooling of the skin. A number of studies have established the critical level of tissue cooling for specific effects. Bugaj (1975) found that ice massage elicits analgesia when the skin has been cooled to and maintained below 13.6°C. Weeks and Travell (1957) tested the anaesthetic effects of ethyl chloride spray and discovered that all subjects experienced anaesthesia when skin temperatures were below 10°C. Cooling below 20°C has been shown to cause a large reduction in the production of the neurotransmitter, acetylcholine, and in the rate of conduction along cooled nerves. Nerve block appears to occur at temperatures below 10°C (Clarke, Hellon, & Lind, 1958). The cooling effect is varied depending upon the size of the nerve fibres, thus resulting in an asynchrony of impulses. It is this blocking and disturbance of nerve conduction to higher centres that is believed to be the major contributing factor in eliciting local anaesthesia (Bugaj, 1975).

Very few studies have evaluated the effectiveness of vapocoolant sprays as a topical anaesthetic in animals. Research with horses has demonstrated safe and effective use of a vapocoolant spray to reduce pain responses associated with arthrocentesis (Fjordbakk & Haga, 2011). Graham *et al.* (1997) found a reduction in peak cortisol response following the use of a topical analgesic spray (Ralgex®) prior to rubber ring tail docking in lambs, however, no significant reduction in pain related behaviour was observed.

We have recently had a study accepted for publication at Veterinary Anaesthesia and Analgesia examining the efficacy of cold-spray anaesthesia for reducing pain response to ear notching and ear tagging in beef calves (Lomax *et al.*, 2015). This study found that a 3 second spray with a topical vapocoolant significantly reduced the peri-operative pain behavioural response to both ear tagging and ear notching in calves. The results indicate potential for use in piglets, particularly as skin thickness in piglets may reduce spray time necessary to induce anaesthesia.

In light of the information provided by previous research, it is well known that castration, tail-docking, teeth-clipping and ear-notching cause significant pain and distress. We also know that while there are effective analgesic and anaesthetic options, these are often costly and time consuming to the producer. It is therefore imperative to devise mechanisms of preventing or minimising pain that are affordable and can be practically applied to production animal husbandry. Based on our previous research into mulesing, castration and tail-docking in sheep (Lomax *et al.*, 2008, Lomax *et al.*, 2010, Lomax *et al.*, 2013), and castration and dehorning in calves (Espinoza *et al.*, 2013, Lomax and Windsor, 2013, McCarthy *et al.*, 2015) we know that we can significantly reduce post-operative pain and improve recovery with the use of a simple, affordable and effective topical anaesthetic product (Tri-Solfen®). We aim to take a multi-modal approach by examining the efficacy of pre-operative cryoanaesthesia to address procedural pain, in combination with topical anaesthesia for post-operative wound anaesthesia. The task now remains to adapt these experimental techniques to the pig industry, in order to provide the Australian industry with a pain-management solution that will improve the welfare and image associated with the industry.

4. Research Methodology

Trials were conducted to assess the efficacy of a topical vapocoolant spray for reducing the pain of ear notching, and of a topical anaesthetic (Tri-Solfen®, Bayer Animal Health Australia) for reducing wound pain following castration. Trials were conducted with institutional ethics approval (Protocol numbers 4905 and 742).

4.1 Topical vapocoolant spray effectively reduces pain of ear notching in neonatal piglets

The aim of this study was to determine whether a vapocoolant spray, comprising of 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, as a model for other invasive procedures.

Temperature validation studies in dead and live tissue were conducted to determine the ability of the vapocoolant spray 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 penetration of the vapocoolant spray. For the study a whole piglet carcass, which had died from natural causes, was sourced from The University of Sydney piggery "Mayfarm" (Cobbitty, NSW, Australia). A K-type temperature thermocouple attached to a temperature data logger (Yu Ching Technology Co. Ltd, Taipei, Taiwan) was validated against a mercury thermometer using a water bath. The water bath was heated to 40°C ± 10°C and temperature maintained using a water bath regulator. To assess temperature conduction of the ear tissue in response to the vapocoolant spray, the K-type thermocouple was inserted (using a 16 gauge needle) beneath the epidermis of the back of the ear to assess penetration through the skin and cartilage. 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 cryo-vac machine, 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 bag, and a the vapocoolant spray was applied from a distance of 10cm (Fjordbakk and Haga, 2011) to the front of the ear at three spray duration times: 1, 2 and 3 seconds. The carcass was re-warmed in the water bath between spray durations. The data logger recorded the change in temperature of the tissue every one second for one minute for each spray duration. Data were uploaded to Excel® via the Temp Monitor S2 Software® (Yu Ching Technology Co. Ltd, Taipei, Taiwan).

Live tissue following successful temperature studies in dead tissue, temperature validation was conducted on live tissue to establish if blood perfusion would affect cooling abilities of the vapocoolant spray. 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 3cm^2 area of the ear was infiltrated with 1mL of lignocaine hydrochloride (HCl, Lignocaine 20, Troy Ilium, NSW Australia) to anaesthetise the tissue. A 16 gauge needle was used as a catheter to insert the thermocouple into the sub-dermal tissue on the back of the ear. The vapocoolant spray was applied from a distance of 10 cm to the front of the ear, 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 one second for one minute for each spray duration. Data were uploaded to 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 vapocoolant spray to induce anaesthesia. This study was conducted at Mayfarm 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 5cm^2 area marked with a scourable marker pen on its left and right side. These areas were used as test sites to determine anaesthetic efficacy of the spray. 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.*, 2007a, Beteg *et al.*, 2011). Each piglet acted as its own control, with each site pinpricked prior to and immediately after spraying with the vapocoolant spray. 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 vapocoolant spray was administered for 2 seconds (determined as optimal from live tissue studies) to each site from a distance of 10cm following initial pinprick, and response recorded.

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 day 1-3 as per 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 to one of four treatment groups (n=10) (Table 1), and blocked across litters to ensure equal representation of each treatment within each block.

Table 1. Treatment groups and numbers of piglets

Treatment	n
Control Positive (POS)	10
Vapocoolant Spray (VS)	10
Lignocaine (LIG)	10
Control Negative (NEG)	10

Treatments POS treatment 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. NEG treatment involved performing the ear notching procedure without an anaesthetic intervention, as per industry standards. Piglets in the lignocaine (LIG) group received 1-1.5 ml of lignocaine HCl (Lignocaine 20, Troy Ilium, NSW Australia) infiltration to the edges of each ear to ensure anaesthesia of the areas to be cut. VS treatment involved application of the vapocoolant spray to the ear notching sites of each ear (the outer ear edges) prior to notching. Spray was applied for 2 seconds at a distance of 10cm, as the optimums established in temperature and tissue validation studies. Spraying did not commence until the piglet was settled (not moving or vocalising) to improve the objectivity of the behaviour score assigned at the time of procedure.

Ear notching Ear notching was performed by the experienced animal house manager, as per 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 (Figure 1).

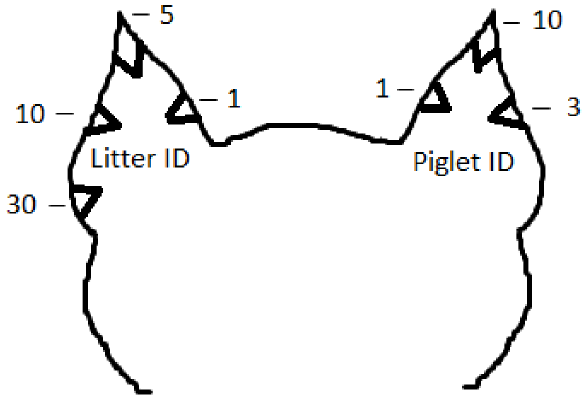

Figure 1. Ear notching sites for litter and piglet ID. Numbers are added through combination of notches to match ID.

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 (approx. 30 seconds per piglet), and stopped once the piglet was released from restraint. Only procedural pain response was assessed, as it was expected there would be no lasting effect of the cryoanaesthesia due to results from tissue temperature validation studies; i.e. once tissue temperature > 10°C, it was expected there would be no anaesthetic effect. Videos were edited prior to scoring to remove the treatment from the videos so only the procedure and animal response was visible to the observer to allow blinding. Videos were numbered for identification 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) reaction, with vocalisation, rapid head withdrawal or body movement considered as a lack of anaesthesia (1).

Statistical analysis Experimental data from the tissue anaesthesia and ear notching trials were analysed using a one-way analysis of variance for general linear mixed models in Genstat (VSN International, 16th Edition, Australia). Binomial distribution (1) was used due to a yes or no response in the experiment. The mean values were then analysed using the Wald test for fixed effects to determine the effect of Treatment, Side, Piglet and Litter, which was deemed significant at the 5 % level of probability.

4.2 *Topical anaesthesia reduces wound sensitivity following castration in piglets.*

The aim of this study is to assess the efficacy of topical anaesthesia Tri-Solfen® applied post-operatively to castration wounds in intensively farmed piglets using wound sensitivity testing.

Animals The trial was performed using 40 Landrace x Large white piglets sourced from a commercial herd located at the 'Mayfarm Piggery', Cobbitty, NSW. Piglets were from mixed litters aged 3-5 day old and weighing at the time of castration. Mean piglet weight was 2.7 ± 0.45kg. The piglets were housed in farrowing stalls with the sow and their littermates before and upon completion of the trial. Animals were removed only for the procedure and subsequent wound sensitivity testing, and were kept in large crates filled with sawdust for the duration of the study (5 hours). A heat lamp was hung over the crate to keep the piglets warm. Piglets were returned to their sows upon conclusion of the study (5 hours).

The trial was conducted over three days, and blocked across six litters with 12 piglets treated on days one and two, and 16 on day three. On each day piglets were weighed and numbered 1-12 (days one and two) or 1-16 (day three) with marker crayon (RAIDL) for identification for the duration of the study (5 hours).

Treatments Piglets were randomly allocated by weight and litter to 1 of 4 treatment groups: (1) Sham castration (SHAM) (*n=10*) – piglets were handled as per normal castration procedure but no incision was made; (2) castration with no anaesthetic intervention (CAST) (*n=10*) – piglets were castrated as per routine husbandry with no anaesthetic intervention; (3) Castration with post-operative topical anaesthesia (TA) (*n=10*) – piglets were castrated as per routine procedure, and 1-2mL of Tri-Solfen® applied during and immediately following castration; (4) castration with pre-operative lignocaine injection (LIG) (*n=10*) – the scrotal site was infiltrated with 2mL lignocaine 5 minutes prior to incision.

Castration was performed by a single trained technician. For treatment piglets were removed from the pen and restrained in ventral recumbency to expose the testes. A small (1-2cm) incision was made using a sterile scalpel into each scrotal sac, and the tunica to expose the testes. The testes were excised using slight pressure on the abdomen and the spermatic cord cut using the scalpel. The procedure took no longer than 1 minute per animal. For TA application 1-2mL of TA was applied directly in to each wound and surrounding cut skin using a sterile 1mL pipette. The pipette was inserted along the spermatic cord, inside the tunica to ensure that all retracted tissue was coated in anaesthetic (Lomax *et al.*, 2010).

Wound sensitivity testing Von Frey monofilaments and an 18G needle were used to determine skin and wound sensitivity pre- and post-operatively as per previous studies (Lomax *et al.*, 2008, Lomax *et al.*, 2010, Espinoza *et al.*, 2013, Lomax and Windsor, 2013). Von Frey monofilaments are devices calibrated to bend at pre-determined pressures and thus are able to provide repeatable stimulation to a site. These are useful in the detection of allodynia (pain response to an innocuous stimulus) and hyperalgesia (an exaggerated response to noxious stimulus), both important in the assessment of pain. Von Frey monofilaments of weights 4g and 300g will be used for light touch (LT) and pain (P) stimulation respectively, at various pre-determined sites in and around the wound(s) elicited from the husbandry procedure(s). The presence or absence of anaesthesia was determined by recording the response to superficial skin pinprick with an 18G needle (Amarpal *et al.*, 2002, Meyer *et al.*, 2007a, Beteg *et al.*, 2011).

Involuntary motor responses were scored using an adjusted customized numerical rating scale (Lomax *et al.*, 2008, Lomax *et al.*, 2010, Lomax and Windsor, 2013). Response was graded by vigour on a scale of severity from 0 to 3. These included local responses at the wound site, such as no motor response (grade 0) minor subcutaneous muscle twitches (grade 1), or partial (grade 2) or full (grade 3) withdrawal reflex. Similar movements of the face and head in response to wound touch stimulus were also recorded and graded by vigour: no discernible movement or reaction (grade 0), eye blink, eye widening, or other minor facial 'awareness' response (grade 1) partial startle response with minor motor movement of the whole head (grade 2) or full startle response with major head jerk and vocalisation (grade 3).
Wound sensitivity was measured at 5 locations, presented from left to right: left cut skin edge, left wound body, the skin in the middle of the incisions (Middle), right wound body and right cut skin edge. Site and von Frey/needle were randomized between each measurement to reduce habituation of response.

For testing, piglets were removed from the pen individually and manually restrained in ventral recumbent by the piggery manager to reduce stress.

Wound sensitivity was measured at 1min prior to procedure, 1min, 30min, 1h, 1.5h, 2h, 2.5h, 3h, 3.5h and 4h post procedure.

Statistical analysis Score data was analysed using restricted maximum likelihood test for linear mixed models (REML) In Genstat® (Version 16, VSNI, UK). Factors assessed for significance included Treatment x time, site and Treatment x vonFrey weight. For interpretation, P values <0.05 were considered statistically significant.

5. Results

5.1 Topical vapocoolant spray effectively reduces pain of ear notching in neonatal piglets

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 10cm to the opposite side of the ear to thermocouple insertion, was adequate to penetrate the ear cartilage and epidermis and reduce the tissue to <10°C (Figure 2).

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

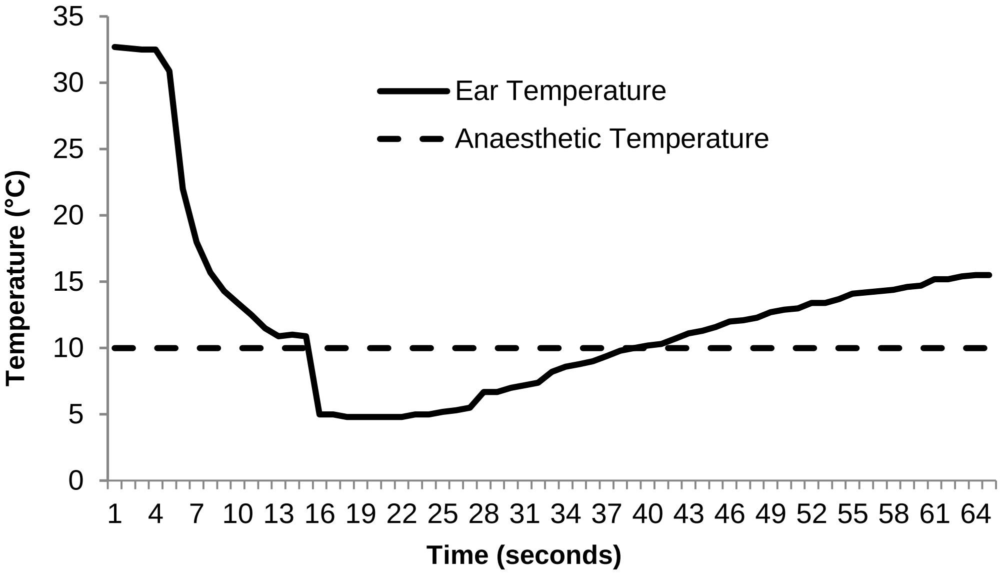

Figure 2. Tissue temperature beneath the epidermis of the back of the ear following a 1 second application of the vapocoolant spray from 10cm in dead tissue. The area beneath the dotted line represents the period of time at which the tissue was at an adequate temperature to inhibit nociception (< 10°C).

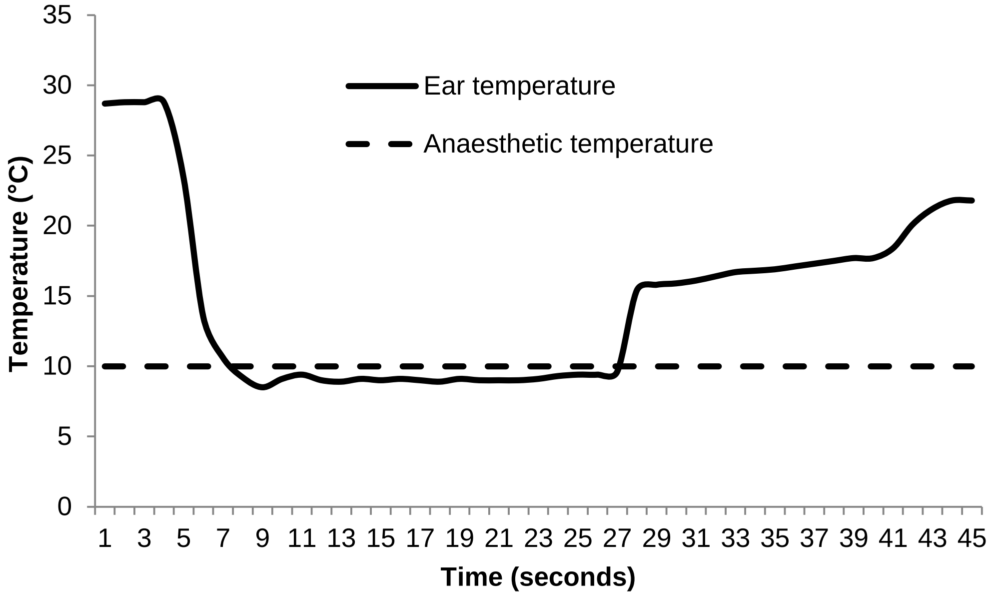

Figure 3. Tissue temperature beneath the epidermis of the ear following a 2-second application of the vapocoolant spray from 10cm to live tissue. The area beneath the dotted line represents the period of time at which the tissue was at an adequate temperature to inhibit nociception (< 10°C).

Clinical validation of tissue anaesthesia The vapocoolant spray significantly reduced nociceptive response to the skin prick test (P<0.001). Piglets were 95% likely to respond to pin prick without the spray, and only 25% likely to respond after the skin was sprayed with the vapocoolant (Table 2).

Table 2. Likelihood of response to pinprick test with 18G needle

Treatment	Response
No Spray	0.95
Spray	0.25
Wald Statistic	26.11
Significance	< 0.001

Effect of a vapocoolant spray on piglet response to ear notching There was a significant effect of treatment on response to ear notching (P<0.001). NEG piglets were 95% likely to respond to ear notching, compared to POS (5%), LIG (5%) and VS (15%) piglets (Table 3). There was no significant difference between responses of POS, LIG and VS piglets (Table 3). All other variables in the trials had no significant effect on responses. The responses exhibited were irrespective of the different litters, piglets and left and right ears (Table 5).

Table 3. Likelihood of response to ear notching. Means with different superscripts within columns are significantly different.

Treatment	Response
Control Positive	0.0500[a]

Vapocoolant Spray	0.1500^{a}
Lignocaine	0.0500^{a}
Control Negative	0.9500^{b}
Wald Statistic	22.73
Significance	< 0.001

5.2 Topical anaesthesia reduces wound sensitivity following castration in piglets.

Time x Treatment There was a significant Time x Treatment interaction (P<0.001). Immediately following castration there was no significant difference in the responses of castrated piglets, however Sham piglets had significantly higher responses to tissue stimulation (Figure 4). Castrated piglets had the greatest response to tissue stimulation from 30min to 4 h following castration. From 2-4 h after castration there was no significant difference between CAST and LIG piglet responses. TA piglets had the lowest mean response scores from 30min after castration until 1h. From 2 to 4h there was no significant difference in the response scores of TA and SHAM piglets.

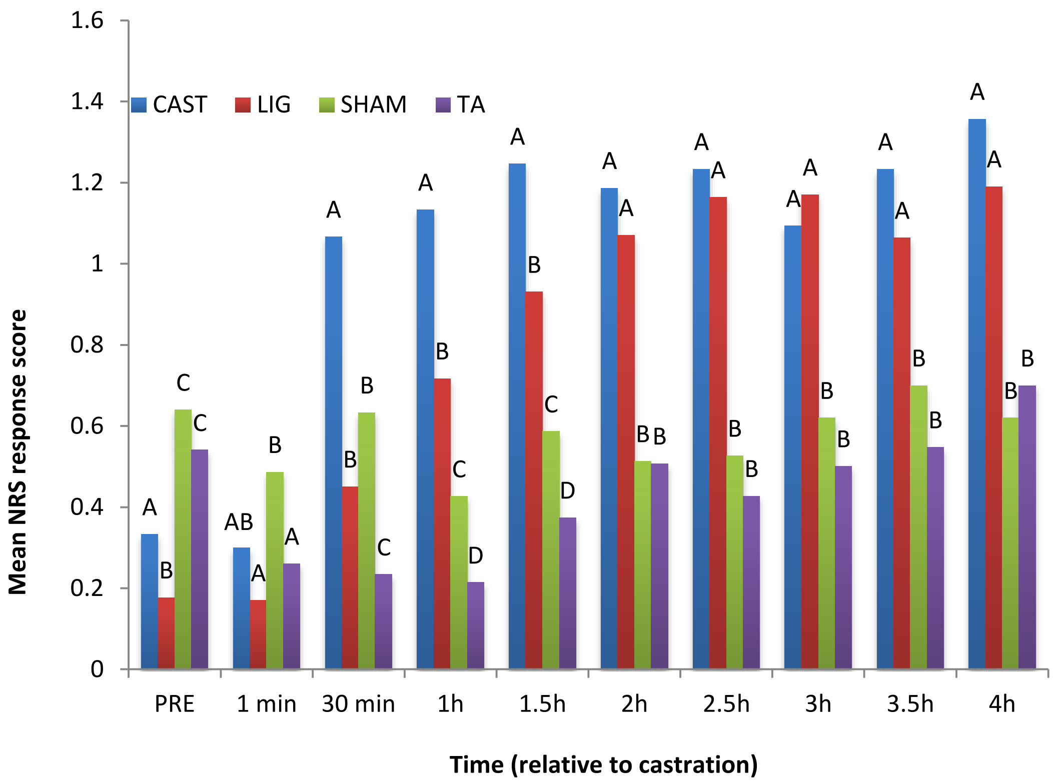

Figure 4. Mean numerical rating scale response score to tissue stimulation (pooled values). Within each time point, values with different superscripts are significantly different (P<0.001).

Treatment x Von Frey There was a significant interaction between von Frey and Treatment (P<0.001). Within each treatment group, responses to each von Frey weight were significantly different. Piglets had the greatest response to needle stimulation, followed by the 300g weight von Frey (Figure 5).

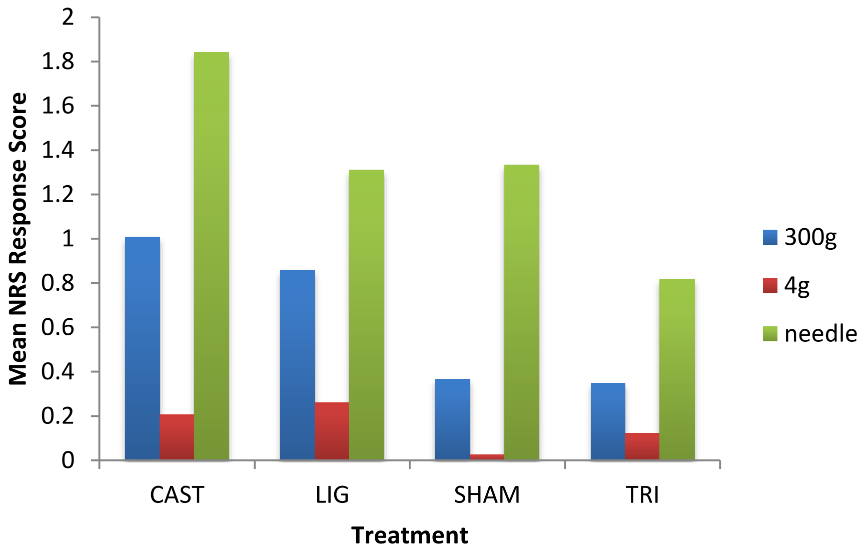

Figure 5. Mean NRS Response score to different von Frey weight stimulations across treatments. Within each treatment all responses differ significantly (P<0.001).

6. Discussion

6.1 Topical vapocoolant spray effectively reduces pain of ear notching in neonatal piglets

This study contributes new information on the use of vapocoolant sprays as local anaesthetics and the potential to induce local anaesthesia in piglet ears. Initial temperature validation indicated that a one second spray was sufficient to reduce the temperature to below 10°C in dead tissue, compared to 2 seconds in live tissue. This was likely due to tissue reperfusion with warm blood.

The validation study found that a 2 second spray reduced tissue temperature to below 10°C for up to 20 seconds. These results align well with similar studies in humans (Travell, 1955, Page and Taylor, 2010). A study on factors affecting the pain of injection in humans found that a 2-second spray to the forearm with vapocoolant spray (halogenated hydrocarbon), was sufficient to cool the skin to well below 10°C (Travell, 1955). However temperature drop in this study lasted only 2-5 seconds, as compared to 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, as cooling can have a differential effect on different nerve fibres (Douglas and Malcolm, 1955). In addition, temperature reduction is less at greater tissue depths, particularly greater than 3cm (Millis, 2004). The depth of tissue of the forearm, in addition to the underlying muscle and greater vascularisation of that area, resulted in rapid recovery of temperature (Travell, 1955). In contrast, the thin, cartilaginous tissue of piglet ears results in slower tissue reperfusion and therefore a longer maintenance of anaesthetic temperature. As ear notching is relatively simple, this duration of anaesthesia (approx. 20 seconds) would allow sufficient time for the operator to perform the procedure.

The vapocoolant spray significantly reduced response to the pinprick test and ear notching. The pinprick test was useful for determining anaesthesia in piglet skin. Piglets were only 25% likely to respond to the pinprick when the area was treated with the vapocoolant spray, compared to 95% with no treatment (Table 2). This suggests adequate cooling and blocking of nerve conduction to induce anaesthesia. Similarly, Travell (1955) used the pinprick test with a 20-guage needle to the skin of the forearm to determine the presence of anaesthesia in treated with a 2 second spray of a vapocoolant (Travell, 1955).

Piglets in the POS, LIG and VS groups were significantly less likely to respond to ear notching than NEG piglets (Table 3). Piglets treated with the vapocoolant spray (VS) had comparable responses to both sham (POS) and lignocaine (LIG) groups. This suggests adequate cooling and blocking of nerve conduction to induce anaesthesia. The rapid action and ease of application of the spray make cryoanaesthesia a practical potential alternative to other pain management options including local anaesthesia. Current pain management options often require administration up to 90 minutes prior to procedure (Earley and Crowe, 2002, Stafford *et al.*, 2002, Ting *et al.*, 2003, Meyer *et al.*, 2007b). Further, in comparison to epidural anaesthesia and sedative analgesics such as xylazine, which may result in delayed return of motor function and hind limb ataxia (Ting *et al.*, 2003, Currah *et al.*, 2009), the brief, localised action of the vapocoolant spray allows farmers to release the calf immediately after the procedure. This is beneficial in terms of time for the farmer as well as stress on an unweaned animal, as restraint and separation from the mother are minimised. Farmers may therefore be more willing to adopt cryoanaesthesia due its practicality and efficacy in reducing the procedural pain.

Application of a vapocoolant spray prior to ear notching induced local anaesthesia and reduced the observed pain response in piglets. When considered as a preliminary model for the investigation of cryoanaesthesia for piglets, efficacy in this model warrants exploration into more invasive husbandry procedures such as surgical castration and tail docking.

6.2 Topical anaesthesia reduces wound sensitivity following castration in piglets

There are increasing economical and ethical imperatives to address pain associated with routine husbandry procedures, such as castration of beef calves. Local anesthetics, such as lignocaine, have been found to reduce acute pain associated with castration when injected into the scrotum (McGlone and Hellman, 1988, White *et al.*, 1995, Jaggin *et al.*, 2006) Regardless, local anesthetic injections are rarely incorporated into routine husbandry procedures of commercial piggeries in Australia.

The development of an affordable and practical means of pain alleviation for such procedures is proposed for incorporation into routine farm management practices. Topical anesthesia, applied during and immediately after the procedure has previously been found to be practical and effective for reducing post-operative pain associated with surgical husbandry procedures in sheep (Paull *et al.*, 2007, Lomax *et al.*, 2008, Lomax *et al.*, 2010, US National Library of Medicine, 2010) and cattle (Espinoza *et al.*, 2013, Lomax and Windsor, 2013). Our studies present evidence that amelioration of pain up to 4h can be achieved for piglets undergoing surgical castration using a farmer-applied, spray-on topical anesthetic. These findings have welfare implications for all piglets undergoing such procedures.

Local anesthetic agents act directly on nerve tissue to inhibit the conduction of nerve impulses responsible for the sensation of pain. They are absorbed through mucosal surfaces and damaged skin, and can effect rapid and profound local anesthesia when applied to open wounds (Brofeldt *et al.*, 1989, Jellish *et al.*, 1999, Kokinsky *et al.*, 1999, Lomax *et al.*, 2008, Lomax *et al.*, 2010). Substance P and bradykinin are chemical mediators involved in the inflammatory response which cause vasodilation, edema and the release of histamine (Ren and Dubner, 1999, US National Library of Medicine, 2010). They can have a slow and prolonged effect, leading to increased sensitization of neurons to nociceptive signals, and exacerbated pain to noxious stimuli. Local anesthetics have been found to suppress bradykinin and substance P-mediated signaling (US National Library of Medicine, 2010). The result is the attenuation of cutaneous micro vascular flare responses in damaged tissue and reduced inflammation, and therefore decreased hyperalgesia of the wound and surrounding skin.

Local anesthesia via injection of lidocaine and bupivacaine has been shown to significantly reduce the cortisol response to surgical castration (McGlone and Hellman, 1988, White *et al.*, 1995). Our results indicate that topical anesthesia can effect a similar reduction in pain. Results indicate that piglets castrated without topical anesthetic treatment, displayed a greater response to wound stimulation than uncastrated or TA piglets. These findings are consistent with observations of piglets receiving local anesthetic infiltration of the spermatic cord and scrotum (Turton, 1962, White *et al.*, 1995). This also supports previous observations of absent or significantly reduced pain responses in lambs and calves treated with TA applied into the castration and tail docking wounds (Lomax *et al.*, 2010, Lomax and Windsor, 2013).

In this study, piglets had the greatest response to needle pin prick stimulation of the tissue within and surrounding the wound. This is as expected as this is an induced known pain response (Amarpal *et al.*, 2002, Meyer *et al.*, 2007a, Beteg *et al.*, 2011). This method was successful at detecting presence or absence of anaesthesia in the wound and surrounding tissues – as sham castrated piglets displayed a response to needle stimulation at all time points.

Castrated piglets that did not receive TA had significantly greater pain responses from 2 to 4 hours following castration. This was expected in CAST piglets as they received no pain management. It is apparent that the lignocaine in LIG piglets had worn off by 2 h time point, as we can see a rise in sensitivity from 30min to 1.5 h, and they do not differ from CAST piglets after 2 h. The TA treated piglets have apparent wound anaesthesia up to 4 h, with significantly lower pain scores than CAST and LIG piglets. This indicates the product is effective at reducing wound pain following castration.

Response scores of castrated piglets were not significantly different immediately following castration (1min). It is hypothesized that this is due to the up-regulation of sensory receptors following the incision which results in a release of natural pain mediators including endorphins to mitigate immediate pain associated with tissue damage. From 30min following castration TA piglets had the lowest pain response scores, indicating effective anaesthesia to wound stimulation.

This study needs further analysis of data to make more clear interpretations of the results. These results will be prepared in manuscript. It is evident from this preliminary analysis that the TA has an effect on wound pain, using wound sensitivity testing as an appropriate means of assessing anaesthetic efficacy.

7. Implications & Recommendations

The topical vapocoolant spray and topical anaesthetic assessed in these studies have significant welfare implications for the industry. The ease of application of both methods of anaesthesia mean that we can provide peri-operative and post-operative pain management simply, effectively and cheaply to farmers. Recommendations are for future work to include larger numbers and combinations of treatments to adequately assess a holistic approach to on-farm pain management. Further, physiological measures should be utilised to objectively quantify presence or absence of anaesthesia.

Further studies assessing specific commercially available topical vapocoolant sprays would benefit the industry.

8. Intellectual Property

No IP has arisen as yet, although the TA product, Tri-Solfen® is currently owned by Bayer Animal Health Australia. Currently, the product is only registered for lambs undergoing mulesing, however it is assumed that results of this study may influence further registration processes. The topical vapocoolant spray has great promise, though there is no particular commercial product recommended. Studies in humans have used a variety of products, all of which would have good application in the industry following further work.

9. Technical Summary

These studies have developed and refined robust methodologies for assessing wound pain and anaesthesia. While methodology for wound sensitivity testing had been previously developed and applied in lambs and calves (Lomax *et al.*, 2008, Lomax *et al.*, 2010, Espinoza *et al.*, 2013, Lomax and Windsor, 2013, Lomax *et al.*, 2013), this study provides new information on its application in piglets. The presence of a significant effect of treatment indicates wound sensitivity was effective at distinguishing between anaesthetic and treatment protocols. This method provides a direct measure for wound pain and presence or absence of anaesthesia. Reliance on behavioural and endocrine methods for assessing pain have major limitations in piglets. Behavioural observations have been used extensively in the literature to determine efficacy of analgesic protocols (Hay *et al.*, 2003, Moya *et al.*, 2008, Kluivers-Poodt *et al.*, 2013). As the current study was assessing efficacy of a topically applied anaesthetic, wound sensitivity allowed the incorporation of behavioural response into a pain assessment protocol for measuring wound anaesthesia. Suggestions would be to include a component of behavioural observations in future to further study effects, however in the limitations of the current study, the two observations could not be combined. This is due to the necessity to re-handle piglets at half hourly intervals for WST, which would interfere with behavioural observations.

Regarding cryoanaesthesia, this study provides new and important information of the application of a vapocoolant spray for inducing tissue anaesthesia to manage peri-operative pain. This method is simple, fast, affordable and effective at reducing pain of ear notching in piglets. Further studies to assess the application for tail docking and castration is warranted, but outside the limitations of the current study. There is potential for commercialisation of the product if wide scale efficacy is seen.

It is concluded that the pilot data collected from this study has been successful at identifying two anaesthetic protocols to address pain of husbandry procedures in piglets. It is recommended that further study into wider application of the techniques and anaesthetic protocols to other husbandry procedures, alone and in combination, should be undertaken.

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11. Publications Arising

We are currently preparing findings from the two studies into publications.