Impact of topical anaesthesia on pain alleviation and wound healing in lambs after mulesing

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Objective To investigate the impact of using the topical anaesthetic preparation Tri-Solfen® on pain alleviation and wound healing in lambs undergoing mulesing.

Design Three separate trials, placebo controlled and/or randomised, were carried out over a 5 month period on three mobs of between 60 and 263 merino lambs undergoing routine mulesing.

Procedure Wound pain was assessed using 10 and 75 g calibrated Von-Frey monofilaments to determine sensitivity to light touch and pain stimulation over a 4 to 8 h period. Pain-related behaviour was documented by trained, blinded observers using a numerical rating scale. Wound healing rates were determined using scaled digital photography and image analysis software to calculate contraction in wound surface area 2 and 4 weeks after mulesing.

Results There was rapid (3 min) and prolonged (up to 8 h) wound analgesia as shown by pain response scores ($P \leq 0.01$), with absent or significantly diminished primary and secondary hyperalgesia ($P \leq 0.01$) and significant reduction in pain-related behaviour ($P < 0.001$) in treated versus untreated lambs. In addition there was improved wound healing in the treated lambs ($P \leq 0.05$).

Conclusion Tri-Solfen® effects rapid and prolonged wound analgesia, reduction in pain-related behaviour and improved wound healing in lambs undergoing routine mulesing, providing effective alleviation of pain associated with routine mulesing in sheep.

Key words: lambs, mulesing, analgesia, welfare
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LT	Light touch sensation
P	Pain sensation
WSA	Wound surface area

Animal husbandry procedures such as mulesing, tail docking and castration are currently performed on between 15 to 20 million merino lambs annually in Australia. These procedures cause acute pain and stress,[1,2] resulting in significant disruption to normal behaviour.[2,3] Despite this, they are currently performed routinely without pain management. Whilst the procedures may be justified for animal preventative health or production management reasons, there is growing opposition to them, particularly from animal advocacy organisations. Increasingly, the infliction of pain is considered to breech acceptable standards of humane animal care. Mulesing, the practice of cutting loose folds of skin from the breech area of sheep, is the most important recent example. Concern for the welfare of lambs undergoing this procedure is resulting in international retail boycotts of Australian wool.

The ideal long term solution is to develop painless alternatives to such procedures. In the interim, the development of effective pain management must be given high priority to decrease animal suffering. At present however, there is lack of commercially available pain-alleviating options that meet the practical and economical constraints of production animal husbandry.

Topical local anaesthesia may be well suited to production animal farming because of its low cost, practicality and ease of application. Local anaesthetic agents generally have poor skin penetrability, which limits their use for pre-procedural skin anaesthesia. However, they are well known to be rapidly and highly effective when applied to open wounds or mucosal tissues[4–9] and may therefore provide an effective means of providing analgesia in the immediate and intermediate post-procedural period, which is arguably the time of maximal pain and stress.[1,2,10–12]

Recently, Tri-Solfen® (Bayer Animal Health, Gordon, NSW) a spray-on topical anaesthetic and antiseptic gel became commercially available for use on farms in Australia. The product was specifically designed for pain management in sheep undergoing surgical procedures such as mulesing. It contains lignocaine (as the hydrochloride) 40.6 g/L and bupivacaine (as the hydrochloride) 4.5 g/L, adrenaline (as tartrate) 24.8 mg/L and cetrimide 5.0 g/L. It is sprayed directly on the wound immediately after the procedure. In this paper we report results from a series of trials investigating the impact of using Tri-Solfen® topical anaesthesia on pain alleviation and wound healing rates, as well as weight gain and mortality in lambs undergoing routine mulesing.

Methods

Three separate studies were performed on lambs of mixed sex from commercial flocks aged 6 to 12 weeks undergoing routine mulesing (study 1) or mulesing and marking (including surgical castration, tail docking and ear notching, plus ear tagging and vaccination) (studies 2 and 3). Lambs were born in both autumn

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and spring of 2006 and were pastured with their dams on two properties, in the southern highlands and central tablelands of NSW respectively.

General management and mulesing

On the day of each trial, lambs from each flock were yarded and drafted into a holding yard. They were then selected at random, weighed, ear-tagged and placed in mulesing cradles. Pre-operative skin sensitivity scoring was performed as required (see below). Lambs were then mulesed by accredited mulesing contractor using standard 'V' modified mulesing technique. In Trials 2 and 3, lambs were also surgically tail docked and ram lambs were surgically castrated immediately prior to mulesing. After this, lambs were managed according to one of three trial protocols in which outcomes were measured at different time points and varying conditions, as outlined below. Outcomes measured included skin and wound sensitivity, pain-related behaviour, wound healing, weight change and mortality.

Assessment of skin and wound sensitivity

Von-Frey monofilaments are used to quantitate sensation. They are calibrated to bend at predetermined pressures to provide repeatable light touch (LT) or pain (P) stimulation. These were used to test skin and wound sensitivity to LT (10N monofilament) and P (75N monofilament) stimulation before mulesing and up to 8 h after mulesing at five predetermined sites on the skin of the breech and nine sites on the mulesing wound (Figure 1A and B).

Evidence of local anaesthesia, allodynia (pain from stimuli such as light touch that is not usually painful), and/or primary and secondary hyperalgesia (exaggerated response to a painful stimulus directly in the damaged tissues or in surrounding undamaged tissues respectively), was assessed at each site. Typical LT and P induced involuntary reflexes and motor responses in the rump and head were graded by vigour. Rump response scores were graded as follows: 0 = no response; 1 = minor involuntary motor response such as local skin twitch, subcutaneous muscle twitch or anal contraction; 2 = partial rump withdrawal reflex such as multiple subcutaneous muscle group contraction and/or lifting of the tail; 3 = full rump withdrawal reflex with lifting of the rump off the cradle. Facial response scores were graded as follows: 0 = no response; 1 = minor facial 'awareness' such as eye widening, blinking or nasal flaring; 2 = partial startle reflex of the head such as slight lifting of the snout or partial head rotation; 3 = full startle reflex of the head, resulting in a major movement such as lifting head off the cradle, full head jerk or full head rotation. Scores for each site were added to achieve a total score for each lamb. Total scores were calculated out of 30 for skin sensitivity around the mulesed area, and out of 54 for direct wound sensitivity.

Pain-related behaviour

Pain-related behaviour was assessed using a numerical rating scale at various different time points after mulesing. The scale was developed based on a combination of previous field

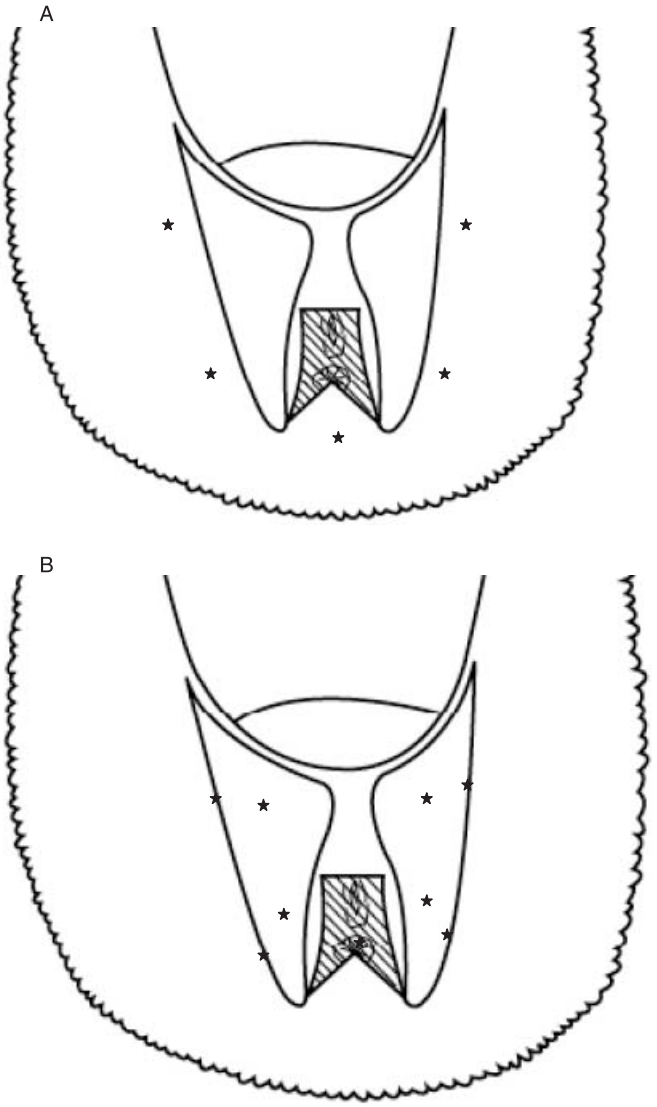

Figure 1. Diagram of sites of light touch and pain stimulation sensory testing. A. On skin of the breech; B. Directly on the mulesing wound sites.

observations and reports of behavioural changes in response to mulesing and/or castration.[1,2] A trained observer (veterinarian or experience sheep handler, 'blind' to the treatment) was asked to observe each lamb for a period of time sufficient to determine posture, gait and evoked behaviour, then grade each lamb on a scale from 0 to 3 where: 0 = no evidence of pain related behaviour; 1 = mildly abnormal posture, gait or behaviours such as mild arching of the back without wide rear leg stance or extension of back legs, ventral lying with legs partially extended, mild stiffening of gait without overt limping or leg dragging; 2 = moderate abnormalities of posture, gait and behaviours such as; statue standing head down with prominent arching of the back, prominent extension and/or abduction of hind legs, marked agitation with leg stamping, ventral lying with hind legs fully

extended, limping or markedly abnormal gait with hind leg abduction and/or dragging, anorexia and lack of interest in feeding; 3 = extreme abnormalities of posture, gait and/or behaviour such as; rear leg collapse, dog sitting, lateral lying or lying with head flat, prominent tremors and shaking, inability to stand and/or marked and unusual leaning.

Wound healing

Wound healing was assessed by mapping wound surface area (WSA) using digital photography and Scion Image PC® digital image analysis software (National Institute Health, USA). Digital photographs were taken with a linear scale held against the wool immediately above the wound. Using the image analysis software, pixels per cm^2 were calculated, then the wound outline was mapped and surface area was calculated in cm^2. Two recordings were made for each image by two separate assessors and the results were averaged to provide the final surface area measurement. Wounds were photographed at the time of mulesing (before application of any treatment) and 14 and 28 days later. Images were identified by lamb tag number and assessors were blind to treatment protocol at the time of performing assessments.

Weights

Weights were recorded at time 0, and day 14 and 28 in Trial 3 using digital scales (Rudweigh®) which were calibrated and zeroed prior to each measurement and accurate to 0.1 kg.

Trial protocols

Trial 1 A placebo controlled trial (n = 60) was initiated to examine skin and wound sensitivity to LT and P sensation 3 min and 4 h after mulesing in lambs that were undergoing mulesing only. Tail docking and castration of wether lambs had been previously performed and wounds were fully healed. Pain-related behaviour was measured 1, 2 and 4 hours after mulesing and wound healing was documented as outlined above. Lambs were managed in 4 sequential groups (15 in each group): Group 1 = mulesing 'control' group (no post mulesing treatment applied); Groups 2 to 4 were treatment groups. Each treatment group received one of three post-mulesing treatments (A, B or C) which were spray-on gels applied by metered dose immediately after mulesing. Treatments A and C consisted of Tri-Solfen® and B was placebo gel. They were applied directly to the wound using the commercially supplied Tri-Solfen® metered dose applicator, in doses of 6 to 12 mLs based on lamb weight, according to manufacturer recommendations for Tri-Solfen®. The placebo was visually indistinguishable from Tri-Solfen® and consisted of the blue spray-on gel base of the Tri-Solfen® formulation with no active anaesthetic or vasoconstrictor ingredients. The three spray-on gels were provided from the manufacturer in identical 1 L containers marked only as A, B or C. After mulesing and wound photography, lambs remained in the mulesing cradle for 3 min after which skin and wound sensitivity scoring was performed. Lambs were then moved in their treatment groups to one of four 20 m^2 pasture covered yards at a minimum of 40 m from the handling yards, for quiet observation and pain-related behaviour scoring. Lambs were then re-yarded and placed in the mulesing cradle for 4-hour wound sensitivity assessment, before being returned to their dams and turned out to pasture. Lambs were re-yarded 2 and 4 weeks later when wounds were examined and re-photographed.

Trial 2 This trial examined wound sensitivity to P stimulation and pain-related behaviour in 80 lambs undergoing both mulesing and marking. The first 24 lambs were allocated to wound sensitivity testing. General management with mulesing was as above. Alternate lambs were treated with Tri-Solfen® or remained untreated. Spinosyn (Extinosad®, Elanco Animal Health, Macquarie Park, NSW) with blue food dye added to achieve blinding was applied according to recommendations as a flystrike preventative treatment. Lambs remained in the cradle for 3 min after which skin and wound sensitivity was performed. They were then housed in a mixed treatment group in an indoor pen and returned to the mulesing cradle at one and 4 h after mulesing for repeat skin and wound sensitivity testing, before being returned to their dams and turned out to pasture. The remaining 56 lambs were identified by a coloured number, and used to assess pain-related behaviour. After mulesing lambs were randomly allocated to receive Tri-Solfen® or remain untreated (28 lambs in each group) and Spinosyn was applied, as above. Lambs were then moved to 3 × 4 m indoor pens for quiet observation and pain-related behaviour scoring. An additional eight lambs served as unmulesed controls. These were placed in the cradle but remained unmulesed and unmarked. Each pen thus contained seven lambs, including six mulesed lambs (mixed treated and untreated) and one handled but unmulesed control lamb. Behaviour scoring was performed 5 min after return to the pen, and at 1 and 4 h. Lambs were then returned to their dams and turned out to pasture.

Trial 3 This trial examined wound sensitivity, wound healing and weight change in a mob of 263 lambs undergoing mulesing and marking. Wound and skin sensitivity to LT and P sensation was assessed in the first 24 lambs with an extra assessment 8 hours after mulesing. General management with mulesing occurred as above. Alternate lambs were then either treated with Tri-Solfen® or remained untreated. Dicyclanil (Clik®, Novartis Animal Health Australasia Pty Ltd, North Ryde, NSW) was used to prevent flystrike (8 mL applied to wool around the wound). Lambs remained in the cradle for 3 min after which skin and wound sensitivity was assessed. They were then kept in a pasture covered holding yard and returned to the mulesing cradle at 4 and 8 h after mulesing for repeat skin and wound sensitivity testing, before being returned to their dams and turned out to pasture. The remaining 239 lambs were weighed, and then each alternate lamb was treated with Tri-Solfen®. Mulesing wounds were photographed prior to application of Tri-Solfen in the first 100 of these lambs. All lambs were treated with Dicyclanil then returned to dams on pasture. Lambs and ewes were re-yarded and drafted 14 and 28 days later. Wounds initially photographed

were re-photographed and lambs were weighed then returned to pasture.

Statistical analysis

Data were analysed using SPSS version 14.0® (SPSS Inc. Chicago, Illinois). Boxplots were examined and one-way analysis of variance was used to measure the short term effects of treatment groups for which there was a reasonable amount of variation. Repeated measures analysis of variance was used to examine within-subject changes over time. Breech skin sensitivity scores before mulesing were adjusted to account for the lower number of testing sites (5) when used for comparison with post mulesing scores from the direct wound assessment sites (9). The average score per site was calculated prior to mulesing and multiplied by 9. Where there were significant differences in baseline measurements ANCOVA/ regression was used to adjust for these differences. Post-hoc pair-wise comparisons are reported for analyses in which the between group comparison was significant at $P < 0.05$. Multiple linear regression was used to examine relationships between contiguous variables.

Results

Weather conditions

In Trial 1, weather was cool to cold and dry at 5 to17 C with no fly activity. In Trial 2, weather was warm and wet, with heavy rain within 24 h with moderate fly activity. In Trial 3, weather was hot and dry at 27 to 33 C with extremely high fly activity, resulting in seven lambs found with fly-strike prior to mulesing, and these were excluded from the trial.

Lamb weights

Mean initial body weight in Trial 1 was 13.1 ± 2.5 kg, Trial 2 was 16.8 kg ± 4 kg and Trial 3 was 14.2 ± 2.6 kg. In Trial 3, weights were recorded Day 0, 14 and 28 on the mob of 263 lambs. Seven flyblown lambs on day 0 were excluded from further recordings, and 14 of the remaining 256 lambs either died or were missing for one or more follow up recordings. Weight data was therefore available for 242 lambs, 121 untreated and 121 Tri-Solfen® treated. There was a mean weight gain of 600 g after 2 weeks (both groups), and 1.3 and 1.4 kg after 4 weeks in untreated and Tri-Solfen® treated lambs respectively.

Morbidity and mortality

There were no clinical signs consistent with lignocaine-induced neuro or cardiotoxicity in any lambs treated with Tri-Solfen®. There was zero mortality in Trial 1. In Trial 2, lambs faced an unexpected major *Haemonchus contortus* burden post mulesing with anaemia and anorexia. Weight and mortality data were therefore not pursued. In Trial 3, 10 of 263 lambs were confirmed to have died during the 4 week trial period (mortality 3.8%). Of these two had been flyblown on day 0, two were Tri-Solfen® treated, two were untreated and four were unable to be identified due to loss of ear tags. In addition seven lambs were missing (three treated, three untreated, one flyblown day 0), presumed to have either died or escaped and mixed into a mob in large adjoining paddock.

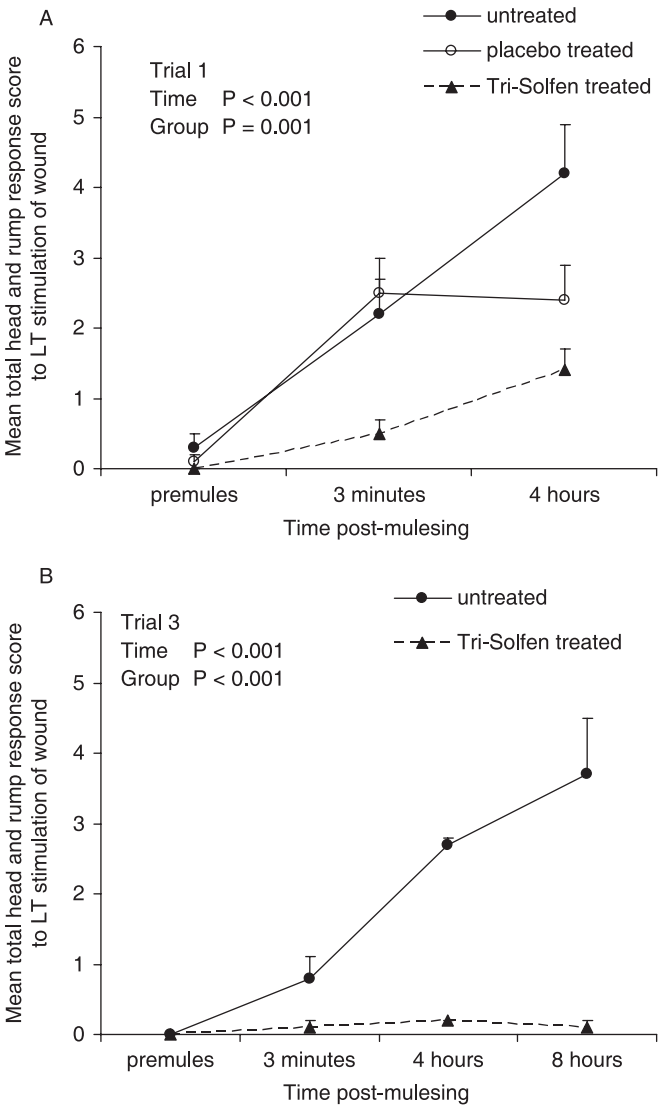

Figure 2. Graphs showing mean total response score (± SE) to light touch of the wound with a 10N Von Frey filament at various time points before and after mulesing. A. Trial 1, with results to 4 h post mulesing; B. Trial 3 with results to 8 h post mulesing.

Response to LT and P stimulation of the wound and surrounding skin

Pre mulesing There was very little response to LT or P stimulation of intact skin of the breech prior to mulesing. Mean response scores from the five testing sites (maximum possible score of 30) were ≤ 0.1 ± 0.4 for LT and ≤ 1.9 ± 3.4 for P, across all three trials (Figures 2, 3 and 4). There were no significant differences between groups within each trial.

Post mulesing Mulesed, untreated sheep. These demonstrated increasing allodynia and primary hyperalgesia with a significant

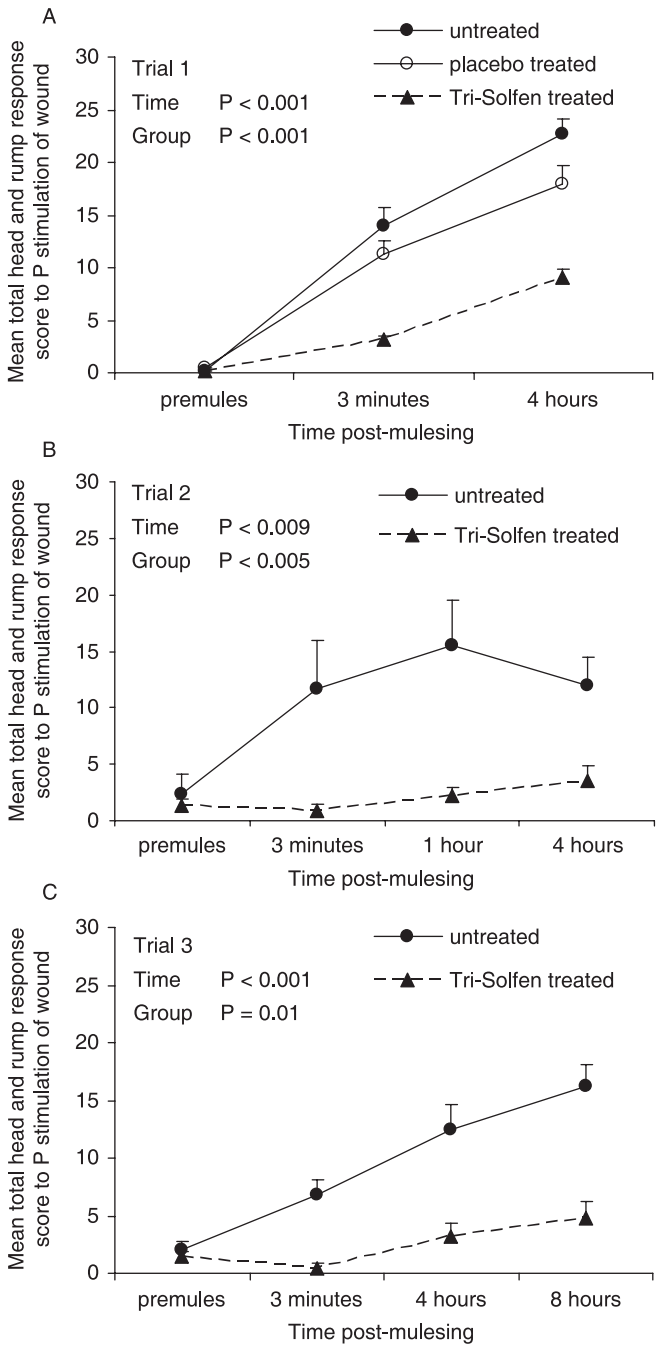

Figure 3. Graphs showing mean total response score (± SE) to pain stimulation of the wound with a 75N Von Frey filament at various time points before and after mulesing. A. Trial 1; B. Trial 2 with results to 4 h post mulesing; C. Trial 3 up to 8 h post mulesing.

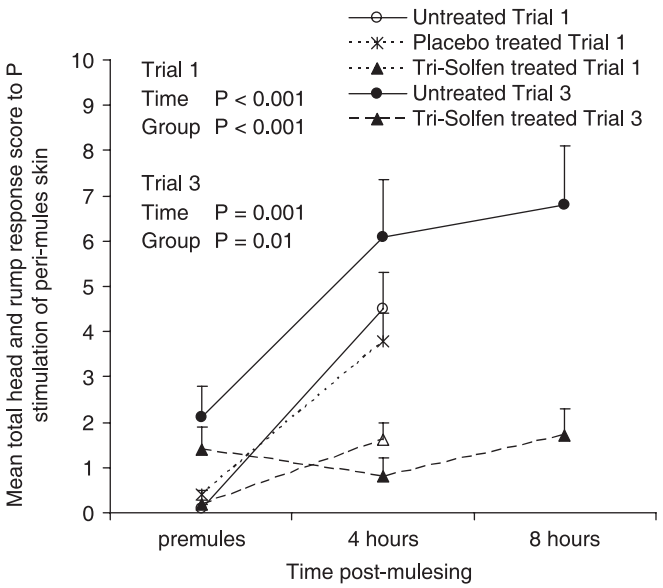

Figure 4. Graph showing mean total response score (± SE) to pain stimulation of the skin around the mulesed area with a 75N Von Frey filament before mulesing and 4 h and 8 h after mulesing.

increase in response over time to LT ($P \leq 0.001$ in Trials 1 and 3, Figure 2) and P stimulation ($P < 0.001$, Trials 1 and 3, and $P = 0.01$ Trial 2, Figure 3). Untreated sheep also demonstrated increasing secondary hyperalgesia, with an increase in response to P stimulation of intact skin around the mulesed area over time ($P \leq 0.003$, Figure 4). Maximal hyperalgesic responses to P stimulation were elicited from the tail and/or the right and left cut skin edges proximal to the tail in all three trials. The central body of the wound distal to the tail was relatively insensitive with maximum mean P response scores on the right and left ≤ 0.7 at all time points in all three trials. Figure 5 show mean response scores to P stimulation at each testing site, at each of the different time points in Trial 3.

Tri-Solfen® treated sheep Primary allodynia, and primary and secondary hyperalgesia were either absent (Trials 2 and 3) or significantly reduced (Trial 1) in Tri-Solfen® treated lambs over the 4 to 8 hour monitored periods. In Trials 2 and 3 there was no significant change in response to LT and P stimulation over time. In Trial 1, LT and P response scores were significantly below those of untreated ($P < 0.001$ for LT, and $P \leq 0.01$ for P) or placebo treated ($P < 0.001$ and $P = 0.002$, respectively) lambs (Figures 2 to 4, Table 1).

Placebo gel treated sheep (Trial 1) The LT responses were similar to untreated sheep and significantly greater than Tri-Solfen® treated sheep ($P < 0.001$) (Figures 2 to 4, Table 1).

Response scores to P stimulation were lower than in untreated sheep ($P = 0.014$) however were significantly higher than in Tri-Solfen® treated sheep ($P < 0.001$). Hyperalgesia to P stimulation in the skin around the mulesed area was similar to untreated sheep and significantly higher than Tri-Solfen® treated sheep ($P = 0.002$).

Pain-related behaviour

Results are summarised in Figure 6 and Table 2. There was a significant increase in pain-related behaviour scores between 1

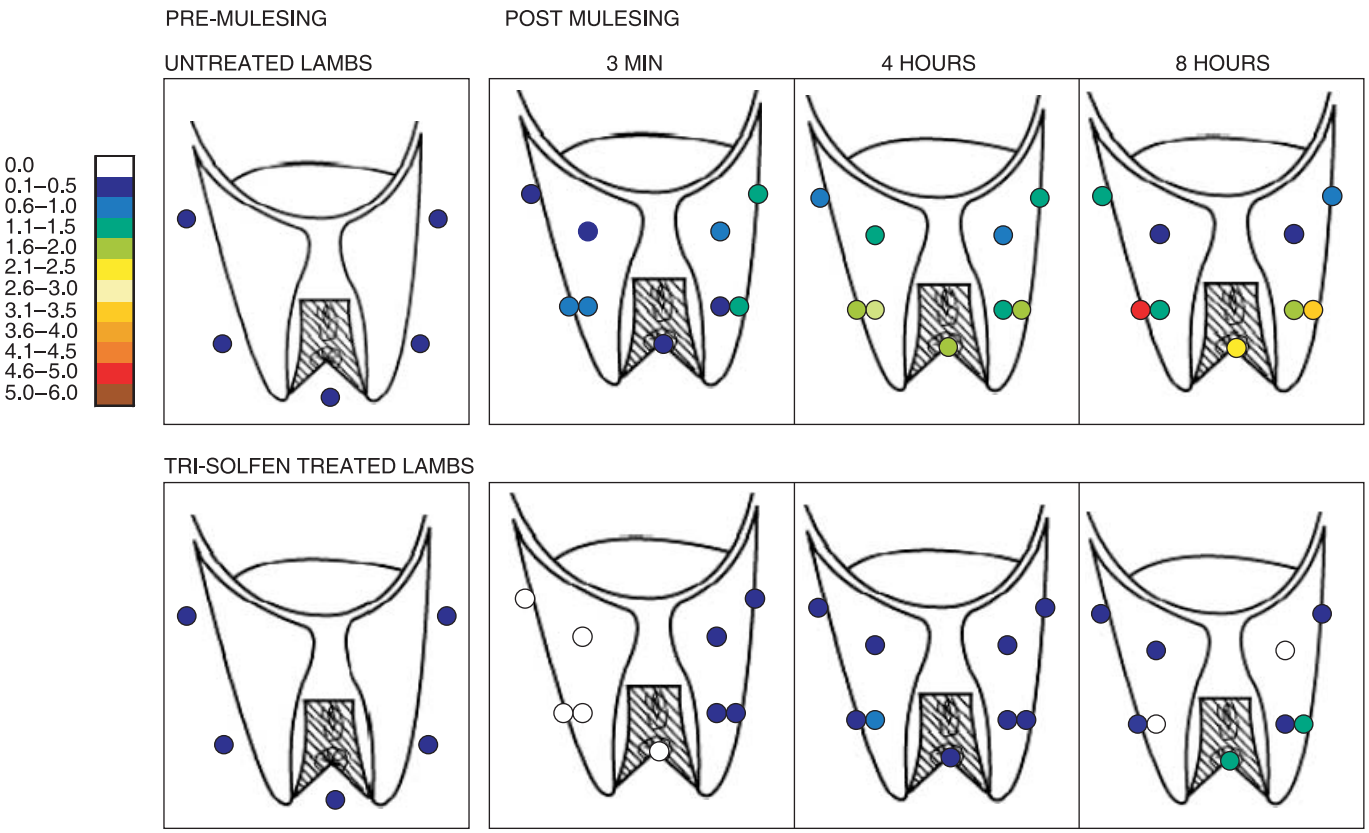

Figure 5. Colour coded representation of the mean motor response score at each testing site to pain stimulation with a 75N Von Frey monofilament, before and at times up to 8 hours after mulesing in Trial 3 lambs, comparing untreated lambs with those treated with Tri-Solfen®.

Table 1. Between subject effects and pairwise comparisons of response to light touch (LT) and pain (P) stimulation of wound and surrounding skin following mulesing comparing untreated lambs with those treated with Tri-Solfen (Trials 1–3) and placebo gel (Trial 1)

	Group 1	Group 2	LT response score wound			P response score wound			P response score Peri-mules skin		
			Mean Diff (1-2)	SE	p group	Mean Diff (1-2)	SE	p group	Mean Diff (1-2)	SE	p group
Trial 1	mules	Tri-Solfen	1.58	0.28	< 0.001	8.10	0.81	<0.001	1.38	0.37	< 0.001
	mules	placebo	0.54	0.32	0.10	2.36	0.93	0.014	0.2	0.43	0.64
	placebo	Tri-Solfen	1.04	0.28	< 0.001	5.83	0.81	<0.001	1.18	0.37	0.002
Trial 2	mules	Tri-Solfen				8.03	2.59	0.005			
Trial 3	mules	Tri-Solfen	1.72	0.22	< 0.001	7.73	1.01	0.01	3.8	0.78	0.012

and 2 4 hours after mulesing (Trial 1), and between 5 minutes and 1 hour after mulesing and marking (Trial 2) with a significant group effect ($P < 0.001$ and $P = 0.055$ Trials 1 and 2 respectively). Tri-Solfen® treated sheep demonstrated significantly lower pain–related behaviour scores compared with placebo gel treated ($P = 0.03$, Trial 1), and untreated mulesed lambs ($P < 0.001$ Trial 1, $P = 0.05$, Trial 2) and were not significantly different from unmulesed controls (Trial 2). Placebo gel treated sheep also had lower pain-related behaviour scores than untreated sheep ($P = 0.01$, Trial 1), although this was less prominent than in Tri-Solfen® treated sheep.

Wound healing

Trial 1 Despite equivalent body weights, mean initial WSA was significantly smaller in untreated than in placebo ($P = 0.002$) and Tri-Solfen® treated ($P = 0.03$) lambs (Figure 7). At day 14 mean WSA was not significantly different between the groups. Using repeated measures analysis there was a significant group

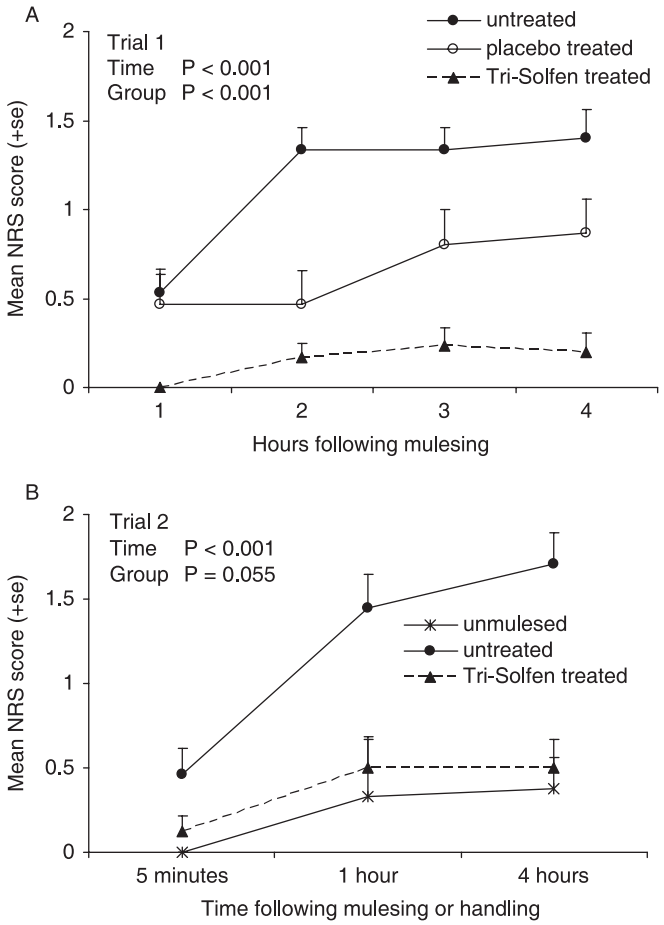

Figure 6. Mean numerical rating scale pain-related behaviour scores at time points up to 4 h following mulesing in lambs comparing untreated lambs with: A. lambs treated with Tri-Solfen® or placebo gel (Trial 1); B. lambs treated with Tri-Solfen® and unmulesed controls (Trial 2).

effect, with placebo-gel and Tri-Solfen® treated groups demonstrating faster wound contraction than untreated lambs over the first 14 days after mulesing (P = 0.05). By day 28, all three groups had ≥ 90% of wounds fully healed (mean WSA < 0.7 cm^2 in all groups). Mean bare area 28 days after mulesing was 20 ± 5 cm^2, and was not statistically different between the groups. There was no significant correlation between lamb weight or initial wound size and bare area size at 28 days. There was no clinical evidence of wound infection or flystrike in any of the wounds during the observed period.

Trial 3 Mean initial WSA was not significantly different between untreated and Tri-Solfen® treated lambs. There was a significant effect of treatment group on WSA measurements over time (P = 0.005). At day 14 and day 28 Tri-Solfen® treated sheep had a significantly smaller average WSA than untreated sheep (P = 0.007 and P = 0.02 respectively, Figure 7). There was positive correlation between lamb weight and WSA on day 0 (R = 0.46, P = 0.001) indicating larger initial wound size in larger lambs. Mean bare area at day 28 was 13.1 ± 5.5 cm^2, and was

Table 2. Between subject effects and pairwise comparisons of pain-related behaviour (mean numerical rating scale scores) following mulesing, comparing untreated mulesed lambs, with: mulesed lambs treated with Tri-Solfen (Trials 1 and 2); placebo gel (Trial 1); or unmulesed controls (Trial 2)

	Group 1	Group 2	Numerical rating scale score		
			Mean Difference (1-2)	Std Error	p value
Trial 1	mules	placebo gel	0.5	0.19	0.01
	mules	Tri-Solfen	0.95	0.19	< 0.001
	placebo gel	Tri-Solfen	0.45	0.19	0.03
Trial 2	mules	control	0.87	0.2	0.001
	mules	Tri-Solfen	0.78	0.2	< 0.001
	control	Tri-Solfen	–0.09	0.2	0.7

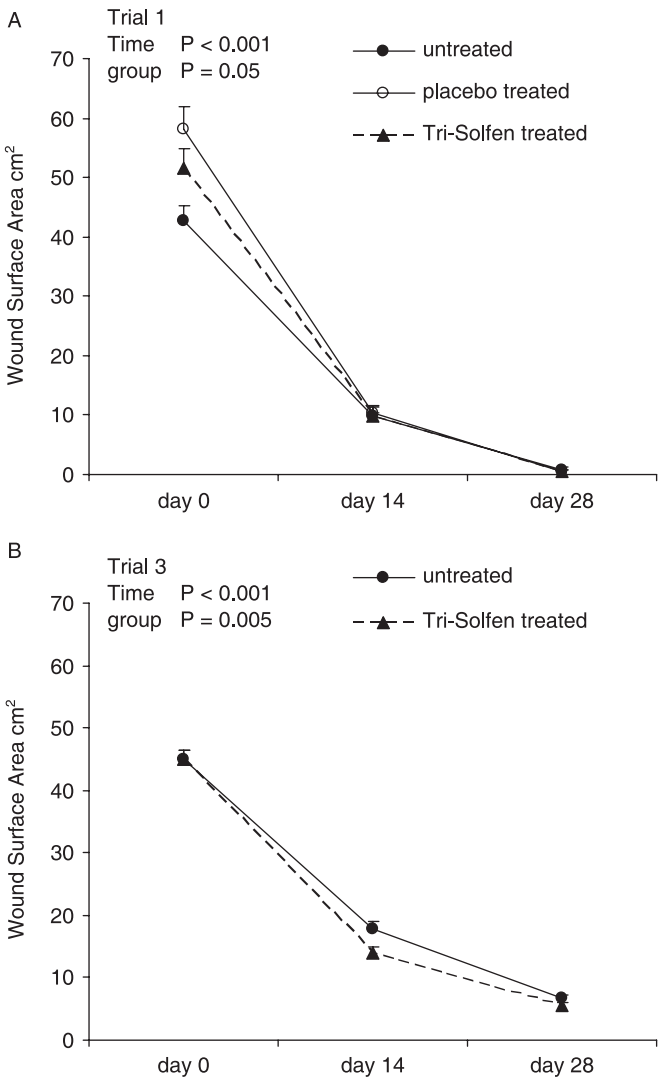

Figure 7. Mean (± SE) mulesing wound surface area (cm^2) in Tri-Solfen® and placebo treated, and untreated lambs immediately after mulesing and 14 and 28 days later.

not significantly different between Tri-Solfen® treated and untreated lambs. Wound contraction was significantly slower in Trial 3 than in Trial 1 lambs.

Discussion

Results from our trials indicate that highly significant alleviation of pain and improved wound healing can be achieved in lambs after mulesing, with and without tail docking, using a commercially available topical anaesthetic and antiseptic spray-on gel preparation. This has a major potential welfare benefit for lambs having this the procedure.

The safety and efficacy profiles of local anaesthetic agents are well described in sheep.[13–16] Lignocaine is highly effective as a local anaesthetic agent when administered using infiltrative techniques in lambs undergoing castration and tail docking,[17,18] however, there is little information regarding the use of topical anaesthesia in lambs. Tri-Solfen® contains lignocaine, bupivacaine and adrenalin in doses equivalent to formulations that have been used to provide effective topical wound anaesthesia and haemostasis in humans.[4,5] The combination of lignocaine and bupivacaine is designed to provide rapid onset local anaesthesia of prolonged duration. The addition of adrenalin is designed to achieve haemostasis and to intensify and prolong the local effect of the anaesthetic actives by reducing systemic absorption. Recently, Paull et al reported a reduction in peak cortisol response and in pain-related behaviour post-mulesing, in lambs treated with Tri-Solfen®,[20] providing the first documented evidence that topical anaesthesia may provide an effective pain-alleviation strategy for lambs undergoing mulesing. These results are supported and enhanced by our own findings.

Documenting the efficacy of pain relief in animals can be difficult, especially in lambs where behavioural responses to pain can be subtle and conflicting. We elected to combine behavioural observations with direct wound sensory testing, rather than measurement of biochemical or physiological responses, as these provide an indirect assessment of pain and are readily confounded by non-pain related variables such as handling, stress and wounding. Cortisol levels, for example, rise during surgical procedures even when pain is completely abolished by general anaesthesia.[21,22] This is because cortisol plays an important role in maintaining blood volume, mediating the inflammatory response and facilitating wound healing so that levels may rise even if pain is absent.[23] This may explain the findings of Paull et al,[20] who reported that treatment of lambs with non-steroidal inflammatory drugs resulted in a significant reduction in post-mulesing pain-related behaviour, but not in a corresponding reduction in cortisol response.

Similar issues limit the reliability of parameters such as heart rate, blood pressure or endorphin release, for estimating pain alleviation in this setting. In addition Tri-Solfen® contains adrenalin, which can have important and confounding effects on such physiological parameters.

Quantitative sensory testing is a validated technique that is widely used in scientific literature. Observation of reflex responses to an acute painful stimulus is an objective, repeatable and readily measurable form of assessing pain and allows the assessor to distinguish between various analgesic interventions.[24] Our findings provide new and important information, particularly regarding the onset, evolution and distribution of pain from mulesing wounds.

Our results indicate that mild hypersensitivity to LT and P stimulation is evident within the wound within in the first few minutes after mulesing. This is followed by increasing allodynia, and primary and secondary hyperalgesia which escalates in the ensuing 8 hours. These findings are consistent with published studies on pain from skin incisions and open wounds.[28–31]

Local anaesthetic agents act directly on nerve tissue to reversibly block conduction of signals responsible for the sensation of pain. By blocking the initial nerve fibre signals local anaesthetics not only effect wound anaesthesia, but can also prevent or reduce the subsequent pain escalation response. This occurs even when local anaesthetics are administered after the incision,[27,29,32] a finding supported by our results.

There appeared to be a moderate pain alleviating effect of the placebo gel. This consisted of the gel base of the Tri-Solfen® formulation without anaesthetics or adrenalin. It is possible that the gel base had an independent intrinsic analgesic effect by forming a barrier over the surface of the wound. Barrier gels and creams have been shown to provide an analgesic effect when applied to open wounds, by coating denuded nerve endings and providing a barrier against on-going environmental exposure and touch stimulation.[33,34]

Another important finding was that the cut skin edge was more sensitive than the body of the wound, particularly in close proximity to the tail. This suggests that the highest nerve fibre density exists in this area and highlights the importance of ensuring adequate cover of the area with the anaesthetic agent.

We examined pain-related behaviour in lambs using a numerical rating scale. These are subjective and can lack sensitivity, but are commonly used for grading pain behaviour[35–37] and have been used in many pain trials.[38–41] We attempted to limit subjectivity and reduce potential bias by using a single observer blinded to treatment protocol and having a clearly defined scale based on abnormal postures and behaviours that have previously been defined in lambs after mulesing.[1,2,11,20] In addition we examined lambs grouped according to treatment (Trial 1) and in mixed treatment groups (Trial 2), and included placebo gel and unmulesed control groups. Our results, indicating that Tri-Solfen® treatment significantly reduced or abolished wound pain and pain related behaviour in the first 4 h after mulesing concurs with and supports the recent findings of Paull et al.[20] Our findings of absent or significantly reduced wound pain in treated lambs 8 hours after mulesing are also consistent with the findings of Paull et al[20] who reported a significant reduction in pain related behaviour (specifically less stiff walking and less standing with hunched posture compared with untreated lambs, and normal feeding) at a similar time point (4 to 8 h post mulesing).

In our trials, pain-related behaviour was greater, and earlier in onset, in Trial 2 than Trial 1 lambs. Observation conditions may have played a role in this finding, as pain-related behaviours may be heightened where animals are held in relatively confined indoor pens as occurred in Trial 2, rather than open paddocks removed from visual and auditory stressors as occurred in Trial 1. However, the finding is most likely to reflect that Trial 2 lambs were also undergoing tail docking and castration. Castration is commonly performed at the time of mulesing and generates significant pain unrelated to the mulesing wound. This pain was not addressed in our trial, and may explain the increased variability in post-mulesing pain-related behaviour scores in Trial 2. To minimise the suffering associated with mulesing and marking it will be necessary to develop strategies to deal with castration-related pain in wether lambs. Studies investigating the efficacy of topical anaesthesia for alleviation of castration-related pain have begun.

Wound healing is a critical outcome of the mulesing procedure. Wound contraction results in reduction of wrinkle and enlargement of the bare area of the breech, which are believed to be the principle factors that reduce susceptibility to fly-strike. Despite this, there is currently very little information documenting wound healing patterns after mulesing and our findings contribute important new information.

Initial wound surface area had an important impact on wound healing rates with larger wounds taking significantly longer to heal within each trial group. Interestingly, and contrary to expectations, initial wound size did not appear to correlate with resulting bare area size in untreated lambs. Together these findings suggest that limiting initial mulesing wound size may result in production and welfare benefits without necessarily having a negative effect on bare area enlargement. This may be an important area for future study.

Studies suggest that local anaesthetic infiltration can have deleterious effects on wound healing after surgical incisions,[42] but impaired wound healing has generally not been apparent in clinical trials.[43] We have documented a significant improvement in wound contraction rates in lambs treated with Tri-Solfen®. In Trial 1, there was an unanticipated and inadvertent discrepancy in initial wound size between groups. Nevertheless improved wound healing with Tri-Solfen® was suggested by finding a significant group effect over the first 14 day evaluation period. This was confirmed in the second larger trial in which initial wound size bias was eliminated. These results support the conclusions of Eroglu et al,[44] that topical anaesthesia does not impair, and may improve wound healing. However, it is probable that actives other than the local anaesthetic agents in Tri-Solfen®, such as the antiseptic and the gel base may be responsible for this effect, as improved healing was also documented in placebo (gel base with antiseptic) treated lambs in Trial 1.

The between-trial wound healing results are interesting in that they appear to conflict with results from a previous study which reported improved wound healing in younger unweaned lambs being mulesed and tail-docked, than in older weaned lambs being mulesed only.[45] Our findings appear to be the reverse. In our trials, lambs being mulesed only (Trial 1) had markedly faster wound healing rates than those undergoing tail docking and mulesing (Trial 3), despite larger initial wound size. We suspect that seasonal conditions, particularly high fly activity, may be an important factor in this discrepancy. The flocks that exhibited the most delayed wound healing, both in our trial and that of Dun and Donnelly,[45] were mulesed during hot conditions with high fly activity compared with their counterparts. Of note, slower healing was associated with higher mortality in Trial 3. These observations suggest that production and welfare benefits may be achieved by controlling the seasonal conditions under which mulesing is performed, and suggest the need for further study of this important observation.

Acute weight loss of up to 10% body weight has been reported in lambs in the first week after mulesing.[1,11] This weight loss was prevented in a group of 21 weaned lambs treated with Tri-Solfen® (unreported data Thompson, Sheil 2005), but not in unweaned lambs treated with Tri-Solfen® in the trial by Paull et al[21] We showed a mean weight gain 2 and 4 weeks after mulesing, with no significant difference between treated and untreated lambs, but no weights were recorded in the first 13 days after mulesing due to the need to minimise wound trauma.

Our finding of absence of clinical cardio and neurotoxicity concurs with other trials reporting the safety of relatively high dose topical lignocaine application to peripheral wound sites, particularly when administered in combination with adrenalin.[6–9,46,47] This is further supported by unpublished data (Thompson, Sheil 2005) that peak plasma lignocaine levels in Tri-Solfen® treated lambs occurred 30 to 60 minutes after mulesing and remained 100 times below toxic thresholds despite application of up to twice the recommended therapeutic dose (n = 12).

In conclusion, the topical anaesthetic and antiseptic formulation Tri-Solfen®, is effective at alleviating pain and enhancing wound healing in lambs post-mulesing. These results suggest that if widely adopted, the use of topical anaesthesia has the capacity to dramatically reduce the burden of acute animal husbandry related pain and suffering in young lambs through-out Australia.

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