A COMPARATIVE STUDY ON WOUND HEALING IN LAMBS AND HOGGETS FOLLOWING MULESING

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Summary

The aims of this experiment were to assess the rate of healing of mulesing wounds based on treatment with a topical anaesthetic (TA), and to compare the rate of wound healing between lambs and hoggets to develop an understanding of possible age effects. Lambs and hoggets were weighed and mulesed (Day 0), with digital photographs of wounds taken at days 0, 14 and 28. Wound surface area (WSA) was then measured using image analysis software. This is a comparative study, comparing data collected from hoggets in 2012 and previously published data on lambs from a trial conducted in 2006. The 2006 component of this trial (published by Lomax et al., 2008) found a significant effect of TA on reducing the rate of wound healing in lambs. The current study did not find a significant effect on wound healing of treatment with TA. However there was a significant difference between age groups, indicating that this is a key area for further investigation.

I. INTRODUCTION

The proposed research aimed to assess the effect of age on the rate of healing of the mulesing wound. It was hypothesised that in older sheep, wound healing time may be reduced due to an increased body size (relative to wound size) and the presence of more subcutaneous fat to protect the open wound (Dwyer, 2008). This hypothesised effect would also be compounded due to the ability of producers to more conservatively, or selectively mules older sheep as with age improved body condition, the breech wrinkle score can reduce (Brown et al., 1966). In this way, the wound size to body size ratio may be reduced even further, as lambs are able to be mulesed based on their specific conformation, rather than on a prediction.

Both Grant (2004) and Lomax et al. (2008) propose that it is in the best interest of the animal if the mulesing cut is as small as possible, whilst still able to provide protection against flystrike. This may be easier to achieve on older lambs as breech wrinkles are minimised as body condition improves.

Due to a larger body size to wound size ratio in older sheep, it was expected that with increasing age, wound healing time would be reduced. It has been shown previously by Lomax et al. (2008) that initial wound surface area (WSA) had a significant effect on the rate of wound healing, with smaller wounds taking significantly less time to heal than larger wounds.

The effect of a topical anaesthetic (TA) TriSolfen® on the rate of healing of mulesing wounds was also investigated. TriSolfen® was licensed for use in sheep in 2006 as a post-operative topical anaesthetic in the form of a spray. Current industry regulations do not require the provision of pain relief post mulesing to sheep under six months of age (CSIRO, 2006), however the adoption of Tri-Solfen® as standard practice by an estimated 70% of Australian farmers shows that welfare is a primary concern - regardless of economic benefit (Windsor & Lomax, 2013).

The research targeted Merino lambs as this breed are predisposed to flystrike due to their heavily wrinkled breech area. The research aimed to determine whether there is an advantage to postponing mulesing from either an economic, welfare or management perspective. As this was a comparative study, with the two trials conducted nearly six years apart, at different properties and using different contractors, there are a large number of variables between the two experiments. For any future investigation into the effect of age on wound healing post-mulesing, it would be ideal to conduct mulesing trials on lambs and hoggets simultaneously, under the same conditions and using the same mulesing contractor.

II. MATERIALS AND METHODS

Animals and housing

A trial was conducted on 200 Merino ewe hoggets aged 10 months at The University of Sydney's 'Arthursleigh' property near Marulan, New South Wales, Australia, in winter 2012. The hoggets had previously been hot-iron tail-docked before 2 months of age with wounds completely healed at the time of experiment. The hoggets were also crutched one week prior to mulesing. Mean weight of hoggets at Day 0 was 27.4 ± 3.5 kg. Experimental protocol was approved by the Animal Ethics Committee of The University of Sydney.

The aims of this experiment were: (1) to assess rate of wound healing based on treatment with and without topical anaesthetic (TA) and (2) to compare the rate of wound healing between lambs and hoggets in order to develop an understanding of possible age effects. Data was collected from hoggets to be compared to previous records on lambs to determine whether there was a difference that should be further explored. The previous data on lambs was published in a paper by Lomax et al. (2008), which employed a similar experimental method, with fewer animals. Details of each trial are outlined in Table 1.

Table 1. Age, sex, number, location and period of data collection of sheep (lambs; hoggets) involved in the assessment of mulesing wounds

Cohort	Age (months)	Sex	Number of animals	Location	Period of data collection
1 (lambs)	1.5	Mixed	100	'Yerilla' (Central Tablelands, New South Wales, Australia)	Spring 2006
2 (hoggets)	10	Female	200	'Arthursleigh' (Southern Highlands, New South Wales, Australia)	Winter 2012

Experimental design and treatments

The cohort of hoggets was divided into two treatment groups, to be compared with data collected from a previous trial on lambs. The treatment groups are outlined in Table 2. Those animals treated with a topical anaesthetic received the immediate application of the topical anaesthetic (TA) gel (Tri-Solfen®) to the entire area of exposed wound.

Table 2. Treatment groups

Cohort	Treatment	Number of animals
1 (lambs)	Mulesing only (M)	50
1 (lambs)	Mulesing with Topical anaesthetic (MTA)	49
2 (hoggets)	Mulesing only (M)	100
2 (hoggets)	Mulesing with Topical anaesthetic (MTA)	100

Wound healing

On the first day of the trial, hoggets were mustered from their grazing paddocks into the shearing shed where they were moved into small holding pens. The hoggets were individually moved through an electronic sheep handler (Hdale Engineering Ltd, Model no: CWC RC) at which time they were weighed. Hoggets were then moved onto an electronic VE-conveyor, ear-tagged, and placed into dorsal recumbency with their hindlegs secured into leg hooks.

Hoggets (total n = 200) were randomly assigned to one of two treatments as they moved through the conveyor, by drawing the relevant number out of a hat: (1) mulesing only (M) (n = 100); and (2) mulesing with the immediate application of a topical anaesthetic (TA) gel (Tri-Solfen®, Bayer Animal Health, Pymble, Australia) (MTA) (n = 100). Sham-mulesing involved manipulation of the breech skin, and making contact with the skin of the breech area using shears, without incising the skin. Mulesing was performed by an accredited and experienced property employee using the standard 'V' modified mulesing technique. Hoggets receiving the MTA treatment were immediately (within seconds) treated with approximately 12 ml of TA over the entire mulesing wound and wound edge, as per industry best practice. Tag number and weight were recorded for each individual animal to allow repeat recording of weight and wound healing over time. Following treatment, animals were released onto the grazing paddocks where they remained until subsequent data was collected.

Wound healing

Wound size was assessed by measuring the surface area of the mulesing wounds of M and MTA hoggets at Week 0, 2 and 4. Wound surface area (WSA) was measured by taking a digital photograph of the wound using a digital SLR camera. A linear scale (30 cm ruler) and animal identification number were included in the photograph. In each photograph, the scale was placed above the wound, against the wool. Image analysis software (Image J 1.47H, Wayne Rasband, National Institutes of Health, U.S.A.) was then used to trace the wound edge and therefore calculate the area of each wound in cm^2 based on the included scale. Using this software, measurements were made for each wound at all time points - by two separate assessors blinded to treatment. Wound areas that varied by greater than 10% we re-measured by both assessors until the areas aligned <10%. The wound area results of both assessors, for each animal at each time point, were results averaged to obtain a final measurement for statistical analysis.

Fig 1. Screenshot of WSA measurement from image analysis program, ImageJ

Statistical Analysis

Wound size data were analysed using an Analysis of Variance approach using statistical program GenStat® (13th Ed. VSN International). For the analysis of wound size, the effect of treatment was investigated. For comparison of lambs and hoggets, age and treatment and their interaction were investigated.

Wound size data were analysed as a difference to initial wound size (Day O) to determine the rate of wound healing.

Data were tested for normality using the Anderson-Darling Test (5%). All data analysed followed a normal distribution and therefore, did not need to be transformed.

For all statistical calculations, P-values less than 0.05 were considered statistically significant.

III. RESULTS

In the comparison between the 2006 lamb trial and 2012 hogget trial, the effect of treatment, age and the interaction between treatment and age were included in the statistical model. The interaction between age and treatment did not have a significant effect ($P > 0.05$) and was therefore removed from the model.

In hoggets, treatment did not have a significant effect on the rate of wound healing, or the percentage change over time. By day 28, wounds treated and untreated healed by 83.95% and 81.64% respectively. In lambs (Lomax et al., 2008), treatment did have a significant effect, with lambs treated with topical anaesthetic demonstrating faster wound contraction at 14 days after mulesing than those untreated ($P = 0.05$).

Table 3. Table of mean percentage change (Day 0-28) by treatment and age

Grand mean	Cohort		Treatment	
	Hogget	Lamb	(MTA)	(M)
86.19	82.84_a	92.55_b	87.18_c	85.15_c

Table 4. Table of mean percentage change (Day 0-28) by treatment and age

		Treatment	
		(MTA)	(M)
Cohort	Hogget	83.95_a	81.64_a
	Lamb	93.32_b	91.8_b

Table 4 indicates that whilst there was no significant difference of treatment on the percentage change in wound size by day 28, there was a significant difference between age groups ($P < 0.001$), with lambs healing significantly faster in the four week period. This is shown in the graph below.

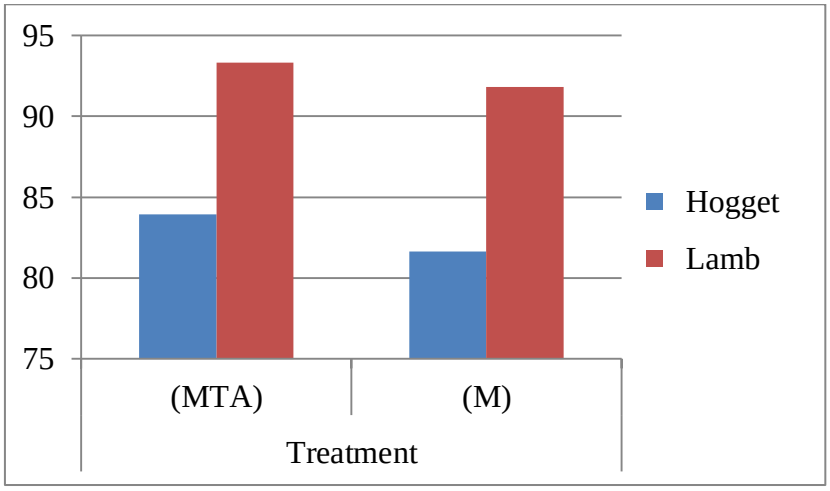

Fig 2. Day 28 percentage WSA reduction by cohort and treatment

IV. DISCUSSION

Both experiments assessed the effect of TA on the rate of wound healing to determine whether the provision of pain relief reduced the time taken for wounds to heal. In hoggets, the provision of pain relief in the form of a topical anaesthetic appeared to have little improvement on the rate of wound healing after mulesing. As outlined in Paull et al (2007), this is likely attributable to the fact that the inflammatory and healing response following a surgical procedure - such as mulesing - is necessary even in the absence of pain.

Anecdotal evidence suggests that older sheep recover better from the mulesing procedure than lambs. It is possible that in older animals, due to a higher plane of nutrition and better resilience to injury, the effects of the topical anaesthetic would be mitigated.

Whilst wound size was measured and rate of healing calculated every two weeks, this analysis looked at the rate of healing as averaged over the whole period of data collection. The primary reasons for wanting to promote rapid wound healing are to improve animal welfare and to prevent infection or contamination from foreign bodies. Lepherd et al. (2010a;b) demonstrated that whilst smaller initial wound size led to a greater degree of contraction in the initial days of healing, the time taken for initial wound contraction was not necessarily an indicator of the time taken for the wound to be completely healed.

When lambs and hoggets were compared, lambs showed a slightly faster rate of wound healing and percentage change over time. This was converse to the hypothesis that hoggets

would have an improved rate of wound healing, due to a decreased wound size: body size ratio. However this could be accounted for due to the smaller wound size in lambs. Mulesing wounds heal by adhesion, meaning that a smaller initial wound size, regardless of body size would require a shorter healing time.

The two cohorts included in this study were mulesed approximately six years apart and the trials were conducted on different properties, with mulesing being conducted by different contractors. The trials were also conducted in different seasons, which could have had an effect on healing time due to either: pasture availability and therefore nutritional status; fly burden or temperature. This introduces a larger number of variables than a closely controlled experiment would contribute. It would be valuable to repeat this experiment, preferably with both cohorts having come from the same flock on the same property, being mulesed at the same time and by the same contractor. This would address the many variables that arise from having two very separate experimental groups. In addition, the number of animals involved in each trial differed, with only 99 lambs in the 2006 trial and 200 hoggets in the 2012 trial. In any future trials, it would be beneficial to have the as large a sample size as possible, with both age groups being as close in number as possible.

Another possible source of error arises due to the reliance on human accuracy when tracing wound images. The tracing of these photographs requires patience and accuracy as the wounds must be traced in finite detail. The implementation of a 10% margin of error meant that many wounds had to be retraced by both assessors, however this was important in order to remove human bias and also to account for any mistakes made during wound tracings.

Whilst this study did have a number of constraints, as a comparative study, it provides a valuable insight into the possible effects of age, treatment and their interaction on the rate of post-operative wound healing in lambs and sheep. It would be extremely valuable to further investigate these effects within one trial to minimise variables and improve the validity of results obtained. The outcomes of this research could provide a scientific basis for the advantages of postponing mulesing - in certain circumstances - for either an economic, welfare or management perspective.

Mulesing has been shown to cause pain, yet the procedure is justifiable for health and management reasons. Production animal welfare concerns are continuously growing and this has the potential to impact the Australian wool industry. In order to meet both industry and consumer expectations, it is necessary to further investigate the effects of age at mulesing and generate a best management approach to the routine husbandry procedure of mulesing. This information can then be used to devise a more effective schedule of husbandry procedures on-farm. The outcomes of this and further research could have a significant impact on areas such as animal welfare, wool industry security, consumer confidence and could lead to economic benefits for wool producers at all levels of the production chain.

V. ACKNOWLEDGEMENTS

The funding provided by the Australian Wool Education Trust (AWET) is gratefully acknowledged. The assistance with data collection provided by the staff from The University of Sydney's Arthursleigh property is much appreciated. The statistical assistance from The University of Sydney's Evelyn Hall is also much appreciated.

REFERENCES

Brown, G., Turner, H., Young, S., Dolling, C., 1966. Vital statistics for an experimental flock of Merino sheep. III. Factors affecting wool and body characteristics, including the effect of age of ewe and its possible interaction with method of selection. Australian Journal of Agricultural Research 17, 557-581.

CSIRO, 2006. Model Code of Practice for the welfare of animals: The Sheep CSIRO Publishing, Primary Industries Ministerial Council.

Dwyer, C.M., 2008. The welfare of the neonatal lamb. Small Ruminant Research 76, 31-41.

Grant, C., 2004. Behavioural responses of lambs to common painful husbandry procedures. Applied Animal Behaviour Science 87, 255-273.

Lepherd, M.L., Canfield, P.J., Hunt, G.B., Thomson, P.C., Bosward, K.L., 2011a. Wound healing after mulesing and other options for controlling breech flystrike in Merino lambs: observations on gross and microscopic wound healing. Australian Veterinary Journal 89, 27-37.

Lepherd, M.L., Canfield, P.J., Hunt, G.B., Thomson, P.C., Bosward, K.L., 2011b. Wound healing after mulesing and other options for controlling breech flystrike in Merino lambs: quantitative and semiquantitative analysis of wound healing and wound bed contraction. Australian Veterinary Journal 89, 61-69.

Lomax, S., Sheil, M., Windsor, P.A., 2008. Impact of topical anaesthesia on pain alleviation and wound healing in lambs after mulesing. Aust Vet J 86, 159-168.

Paull, D.R., Lee, C., Colditz, I.G., Atkinson, S.J., Fisher, A.D., 2007. 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. Australian Veterinary Journal 85, 98-106.

Windsor, P.A., Lomax, S., 2013. Addressing welfare concerns in control of ovine cutaneous myiosis in sheep in Australia. Small Ruminant Research 110, 165-169.