Abstract

Aims: To evaluate the success of a commercially available analgesic device (CoolSense; Coolsense Ltd, Tel Aviv, Israel) in ameliorating pain while sampling from subcutaneous tissue cages in sheep.

Methods: The CoolSense device was used, as part of a major parent study involving repetitive percutaneous sampling of subcutaneous tissue cages in seven sheep. Sampling was performed by passing a hypodermic needle through the skin and withdrawing fluid from the tissue cage. Each sheep had 10 tissue cages that were individually sampled 14 times over 74 hours. The device was placed on the skin of the sampling site immediately before sampling cooling and numbing the skin. The reaction of the sheep was observed by the operators, flinching or jumping as the needle was passed through the skin was deemed to be a failure. We recorded the success or failure of the device for each needle stick. This was opportunistic data collection as part of a pharmacokinetic trial therefore no controls were included.

Results: A total of 1655 observations were recorded and then analysed using a generalised linear mixed model. Overall, 1380/1655 (83.4%) observations were recorded as successfully providing analgesia. Marked inter-occasion variability was noted with success ranging from 61.42% to...
92.86% across sheep:period (approximately 140 observations each). As no controls were available the effect of treatment could not be evaluated.

**Conclusions and Clinical Relevance:** The CoolSense device is a viable option for veterinary research and clinical applications.

**KEYWORDS:** Analgesia, Cryogenic, Pain, Research Model, Sheep

**GLMM**

**Generalised linear mixed model**

**Introduction**

Biological research samples such as blood, fine needle aspirates, synovial fluid, and interstitial fluid are often obtained by percutaneous aspiration utilising a hypodermic needle and syringe (Silber *et al.* 2010; Pelligand *et al.* 2014). Passing a needle through the skin causes transient pain or discomfort, and repeated sampling leads to an increased cumulative animal welfare burden (Weary *et al.* 2006), especially if not mitigated by effective analgesia.

Ethics approval of repeated sampling studies should require the provision of skin desensitisation or analgesia, due to the high cumulative burden imposed by repeated sampling. Several options might be considered including topical local anaesthetic cream, subcutaneously injected local anaesthesia, lidocaine transdermal patches, and capsaicin cream. These options, however, generally provide only minimal or short-duration analgesia. Placing lidocaine transdermal patches around the sampling site would overcome these problems but it is likely to fail in that the patches often fail to adhere well to the skin of many species.

Whilst planning a pharmacokinetic study requiring multiple, repeated percutaneous fine needle aspirations in sheep, we chose to test a commercially available analgesic device (CoolSense; Coolsense Ltd, Tel Aviv, Israel). Although the device has been shown to be effective in reducing needle-stick pain in humans (Ragg *et al.* 2017; Suami *et al.* 2019) there was no reported evaluation of the CoolSense device in veterinary species.

Therefore, this manuscript aims to describe the use of the CoolSense device for the provision of analgesia during percutaneous sampling in sheep.
Materials and Methods

CoolSense devices were obtained from a commercial medical equipment supplier (Team Medical Supplies, NSW, Australia). The devices are designed to provide short term dermal anaesthesia to the area of application by rapidly cooling the skin. The device is kept in a -20°C freezer until immediately before use. The metal pin is coated with isopropyl gel to prevent thermal burns and held on the skin for a short period of time. A coloured light system shows if the device is below, at, or above the operating temperature.

Seven merino wethers, approximately 18 months old and ranging from 39-59 kg, were enrolled (University of Melbourne Animal Ethics approval 1814590). Each wether was determined to be healthy by clinical examination and routine haematological and biochemical testing prior to enrolment. All sheep were housed in a corrugated iron shed on slatted floors with water supplied ad libitum. Pellets (Sheep & Cattle Rumevite, Townsville QLD Australia) and lucerne chaff were provided daily. Ventilation was provided by passive air movement through doors and windows, and experiments were conducted between April 2019 and August 2019 in Werribee, Victoria, Australia.

To prepare the animals, general anaesthesia was induced by administering midazolam (0.5mg/kg IM, Hypnovel Pharmaco Australia, Gordon NSW), followed by lignocaine (2mg/kg IV, Lignomav Mavlab, Logan City QLD) and thiopentone (Thiobarb, Jurox Rutherford NSW) IV to effect. Following endotracheal intubation, the anaesthesia was maintained with isoflurane in oxygen, using a rebreathing system. Subcutaneous administration of lignocaine numbed the surgical area. Five hollow silicone cylinders were then implanted subcutaneously on each side of the neck of each wether to form ten tissue cages. For post surgical analgesia, 10mg/kg paracetamol was administered IV during surgery and 15mg/kg orally every 12 hours for six doses postoperatively.

A randomised cross-over two-phase pharmacokinetic study was conducted three and seven weeks after implantation of the tissue cages, to evaluate a single intravenous administration of 4 mg/kg carprofen (reported elsewhere). The wool over the tissue cages was shorn using handheld clippers to allow visualisation. As a component of the pharmacokinetic study during one of the phases, 1 mL of 1% κ-carrageenan was injected into the five cages on a single side, causing mild inflammation. Samples were collected from each of the ten tissue cages 14 times during the 74 hours post-dose. These samples were obtained by inserting a 20g hypodermic needle through the skin directly into each tissue cage and aspirating ~1.5ml of fluid. Each set of 10 samples took approximately 15 min to collect.
As an observational study, independent of the pharmacokinetic study, the response outcomes from the use of the CoolSense device were recorded for each needle stick at each time point in all but the 3-week sampling for two sheep. In total, 140 needle sticks were performed in each sheep for each sampling period.

A total of six CoolSense devices were used. The devices were placed in a freezer at -20 °C overnight prior to the sampling period. During the sampling periods, the devices were stored in a portable freezer on wet ice, near the sheep pens, to allow rapid rotation of devices as needed. Before each use, the temperature was confirmed to be correct (-4° C to 0° C) using the inbuilt light system and alcohol gel was applied to the pin in accordance with the manufacturer's directions. The alcohol-containing cap was replaced once exhausted, as needed. The device was held on the skin overlying the sampling site for 20 s immediately before each tissue cage was sampled. The needle was passed through the skin at the location of the device application which was visible as a slight depression in the skin. As the needle passed through the skin, the reaction of the sheep was monitored by two observers, both experienced veterinarians. The first observer physically restrained the sheep and collected the samples by passing the needle through the skin. The second observer remained outside the pen. If the sheep flinched or jumped as the needle was moved through the skin, a failure was recorded, while a success was recorded otherwise. An outcome was recorded for each use of the device.

**Statistical Analysis**

A generalised linear mixed effects model (GLMM) was used for the analysis of the binary (success or failure) outcome data. This model was used to estimate the influence of predictors on the probability of success and the baseline probability. As no sham controls were included in the data, an effect of treatment could not be estimated. The analysis may also allow the identification of individual “responders” for given occasions. Explanatory variables such as side (left or right), size of the tissue cage (cm, median centred) and the presence of carrageenan in the cage (yes or no) were included as fixed effects with the coefficients applied equally across all outcomes. In order to account for individual subject variability during each sampling period and variability over time within a sampling period, random intercept variability was included in the model with terms for sheep-period (n=12) and time-point within sheep-period (n= 168). Between-sheep variability was not estimated as the data was not sufficient. The logistical regression model used maximum likelihood function. Marginal and conditional $R^2$ (Nakagawa et al. 2017) was estimated using the 'muMin' package (Barton 2019), 95% confidence intervals were determined from the profile likelihood (Cole et al. 2013).
**Equation 1:** \( \logit(\hat{Y}) = \beta_0 + \beta_1 \cdot \text{Side} + \beta_2 \cdot \text{Size} + \beta_3 \cdot \text{Carrageenan} + (1|SP_i) + (1|\text{SPT}_j) \)

Where \( \hat{Y} \) is the predicted success probability, 1 is the model intercept, \( \beta_{0-3} \) is the estimated coefficient for the respective fixed effect, SP\( _i \) is the random effect for subject-phase \( i \), and SPT\( _j \) is the random effect for subject-phase-time \( j \).

**Results**

Overall, the device was simple to use, with multiple operators being able to use it by following the manufacturers included instructions. Multiple (10) samples were taken in rapid succession at each timepoint. Whenever the CoolSense temperature indicator remained green, the same device continued to be used for sampling series, typically 2 devices would be used for each set of 10 cages. This exceeded the recommended use pattern limit. Despite these conditions, the only observed device breakdown was the exhaustion of the light battery.

No adverse events were observed from the use of the CoolSense device. A result was recorded for each of 1655 samples. Results were recorded for both sampling periods for five out of seven sheep while the remaining two have records for the only second period. Overall, 1381/1655 (83.4%) of the recorded samples were deemed to be successful. By sheep and period (~140 samples each) the success proportion ranged from 61.42% to 92.86%. The estimates for intercept, random variables and fixed variables generated by the GLMM are displayed in Table 2 as log odds. The model explains little of the observed variability with a maximum \( cR^2 \) of 0.22. The mean estimate of the intercept (baseline probability without predictors) predicts a probability of 0.79 with a 95% CI between 0.71 and 0.86. The fixed effect parameters (cage size, side, and carrageenan) have a negligible effect on the overall estimate of success. A marked inter-occasion variability is seen, with the sheep-period odds 95% CI ranging from 4.5 to 17.7 (Probability=0.82-0.95). When the time point within individual occasion is considered, the 95% CI for the odds ranges from 2.9 to 12.9 (P=0.74-0.93), the models’ predictions on the between occasion variation are shown in figure 1 in success probability. The study design confounds the variables, size and side: all sheep had the same layout of cages, and the samples were taken in the same order.
Discussion

To the authors' knowledge, this is the first reported use of the CoolSense device in animals. Other reports have shown it to be effective before intravenous catheterisation in children (Ragg et al. 2017), as well as for procedures involving intradermal injections (Suami et al. 2019).

As researchers, we have an obligation to minimise the welfare burden imposed on research subjects. As the pharmacokinetic study had a high cumulative burden from repeated needle sticks, effort to provide skin desensitisation was required. When the transdermal lignocaine patches failed other options including subcutaneous injection of local anaesthetic, topical anaesthetic creams and capsaicin cream, were discussed. Re-application and waiting for the onset of effect would interfere with sample timing for the pharmacokinetic study. The CoolSense offered a rapid and repeatable option that would not interfere with the pharmacokinetic study. The CoolSense device was designed for the mitigation of needle induced pain and discomfort.

A limitation of this study might be that CoolSense efficacy was judged by subjective criteria. However, needlestick pain is transient in nature and is not expected to produce long-lasting changes in behaviour or physiological surrogates for pain. For example, ethograms have been validated for the use in sheep (McLennan et al. 2016) and other species (Hielm-Björkman et al. 2011; Gleerup et al. 2015; Reid et al. 2017) for both acute and chronic pain but these rely on the pain continuing for a least some time. Biochemical measurements such as cortisol from plasma or saliva are unlikely to be sensitive or specific enough to distinguish between each of the ten needle sticks within a sample time point and to distinguish needle sticks from handling stress. Cortisol rises slowly in comparison to the duration of a needle stick and has been shown to miss the first few minutes of pain in surgical interventions (Mellor et al. 1997). An extremely rapid half-life would be needed for a biochemical measurement to return to baseline between each needle stick (<1.5 min between needle sticks), this would then create significant logistical issues in collecting the sample in the required time frame. Physiological plasma or saliva measurements would require a further 9-10 samples to be collected at each time point, this may increase the welfare burden on the animal. Infra-red eye temperature measurements have been used in cattle (Stewart et al. 2008) to measure acute surgical pain but its results in sheep have not been reliable or sensitive (Stubsjøen et al. 2009; Sutherland et al. 2020). Infra-red thermography is also constrained by the high cost of equipment. In this experiment, multiple sheep were being sampled simultaneously, and multiple cameras would be needed. Human studies have relied on post-procedure questionnaires of the patient and operator (Mahshidfar et al. 2016; Ragg et al. 2017) and the current study takes a similar approach to the measurement of the outcome. Overall, needle stick pain is well recognised but poorly defined or measured, and
therefore we chose to use a binomial variable in preference to a graded scale. Further studies in this area would likely need to develop and validate a system for measuring the pain and discomfort from needle sticks.

As part of the pharmacokinetic study, the sheep received carprofen which is a known analgesic agent. Carprofen is a non-steroidal anti-inflammatory drug that produces analgesia by reducing the formation of inflammatory mediators via cyclooxygenase 2. It would not be expected to reduce the nociceptive signal transmission of the needle stick, although it may reduce hyperalgesia from repeated sticks.

The data presented in this report are constrained by the opportunistic nature in which they were collected. Therefore, no controls or sham applications could be included in the study design. This is the major limitation of these data, as the proportion of failure that would be seen without the device is unknown. Sham applications should be included in future studies and would allow the effect of treatment to be estimated. Although the devices were used by six operators, insufficient data were collected to examine the effect of the operator on the outcome. The operators were not blinded to cage size, location, or carrageenan, and the samples were collected in the same order on each occasion. These decisions, and associated workflow, were determined by the pharmacokinetic study design to ensure samples were correctly identified.

The results shown indicate an acceptable level for the mitigation of short acute pain from a needle stick. The predictors in the model failed to explain the majority variability in success rate, suggesting other users can expect a similar success rate at any timepoint, without the need to identify individual responders. Overall, the device appeared to be useful for providing analgesia and reducing the cumulative burden on research animals undergoing multiple needle insertions, with no observable adverse effects and little risk of interference with study aims. We conclude that the CoolSense device has merit for further research and clinical applications in veterinary medicine and further investigation should be conducted.

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Conflict of Interest Statement
Declarations of interest: none
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Table 1. Observed successes using CoolSense to minimise pain perception during percutaneous needle-stick sampling for each sheep in each period expressed as the proportion (percentage) of samples deemed successful.

<table>
<thead>
<tr>
<th>Sheep</th>
<th>Period 1</th>
<th>Period 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-</td>
<td>113/131 (86.3%)</td>
</tr>
<tr>
<td>B</td>
<td>-</td>
<td>116/135 (85.9%)</td>
</tr>
<tr>
<td>C</td>
<td>111/139 (79.9%)</td>
<td>C</td>
</tr>
<tr>
<td>D</td>
<td>115/139 (82.7%)</td>
<td>112/137 (81.8%)</td>
</tr>
<tr>
<td>E</td>
<td>118/139 (84.9%)</td>
<td>C</td>
</tr>
<tr>
<td>F</td>
<td>115/138 (83.3%)</td>
<td>124/140 (88.6%)</td>
</tr>
<tr>
<td>G</td>
<td>130/140 (92.9%)</td>
<td>C</td>
</tr>
<tr>
<td>Total (Mean% [CV%])</td>
<td><strong>589/695 (84.7% [5.77])</strong></td>
<td><strong>563/694 (81.1% [14.3])</strong></td>
</tr>
</tbody>
</table>

C denotes presence of carrageenan in half of the cages for that sheep-period.
Table 2. Coefficient Parameter Estimates (in log-odds scale) of fixed and random effects as estimated in the generalized mixed model. (3 s.f.)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MLE</th>
<th>2.5% CL</th>
<th>97.5% CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.32</td>
<td>0.893</td>
<td>1.79</td>
</tr>
<tr>
<td>$\sigma$, Sheep: Period</td>
<td>0.696</td>
<td>0.615</td>
<td>1.09</td>
</tr>
<tr>
<td>$\sigma$, Sheep:Period:Time</td>
<td>0.168</td>
<td>0.160</td>
<td>0.768</td>
</tr>
<tr>
<td>Tissue Cage Size (cm)</td>
<td>0.0426</td>
<td>0.0166</td>
<td>0.0692</td>
</tr>
<tr>
<td>Tissue Cage Side (R)</td>
<td>0.213</td>
<td>-0.0661</td>
<td>0.496</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>0.155</td>
<td>-0.212</td>
<td>0.526</td>
</tr>
</tbody>
</table>

MLE: maximum likelihood estimate.
CL: confidence limit.
Figure 1 – Histogram of fitted probabilities from the GLMM. Showing the estimated probability density (y-axis) of the probability of successful analgesia from the CoolSense device during needle sticks in sheep, for within a given timepoint within a subject-occasion (x-axis). A success proportion between 70% and 95% would be expected in most timepoints.
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