The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK
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Evaluation of euthanasia of sheep with intravenous saturated salt solutions to enable the collection of whole, intact brains

KJ Stanger*†, NJ Kells‡, AD Fisher§, T Jubb#, J-L Rault¶ and C Johnson¥

† University of Melbourne, Faculty of Veterinary and Agricultural Sciences, 250 Princes Highway, Werribee, VIC 3030, Australia
‡ Animal Welfare Science and Bioethics Centre, Massey University, New Zealand
§ Animal Welfare Science Centre, University of Melbourne, Australia
# Livestock Health Systems Australia, Bendigo, Australia
¶ Institute of Animal Welfare Science, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Austria
¥ Institute of Veterinary, Animal and Biomedical Science, Massey University, New Zealand
* Contact for correspondence: Kelly.stanger@unimelb.edu.au

Abstract

Captive bolts or firearms are unsuitable for euthanasia of livestock when an intact brain is required for diagnostics. Injectable barbiturates can be used, but this method carries risk of poisoning animals eating the carcase. Intravenous saturated salt solutions have been used to euthanase heavily sedated ruminants and are cheap, readily available and not a risk to scavenging animals. However, there is concern that they may be painful or cause distress to animals that are not unconscious. This study aimed to determine the suitability of saturated salt solutions, in combination with xylazine, as a method of euthanasia of ruminants using a sheep model. Thirty-two sheep were sedated with xylazine (0.4 mg kg^{-1} IM) and euthanased with an intravenous overdose of pentobarbitone (PENT; n = 10), saturated potassium chloride (KCL; n = 11) or saturated magnesium sulphate (MGS; n = 10). Time until end of rhythmic breathing and cardiac arrest, and movement events were recorded. Conscious perception of pain was evaluated by measuring cortical brain activity by electroencephalography (EEG). There was no evidence of perceived pain or unpleasant sensory experience for any treatment as indicated by P50, P95 and P_{tot}, and so all methods were deemed humane. Time until transient EEG was comparable for all treatments. Time until onset of isoelectric EEG was prolonged for KCL. Animals euthanased with KCL consistently exhibited severe reflex movements during infusion (eg kicking, convulsion). No severe movement events were observed in animals euthanased with MGS, hence, physiological and movement data support the preferential use of MGS over KCL.

Keywords: *animal welfare, electroencephalogram, intravenous euthanasia, magnesium sulphate, potassium chloride, ruminant*

Introduction

In Australia, the recommended method of euthanasia of ruminant livestock is captive bolt or firearm (Animal Health Australia [AHA] 2016a,b). These devices provide a rapid and humane means of euthanasia but may not always be available. Further, the use of firearms and captive-bolt devices may not be appropriate when whole brains are required for diagnostic investigation. In Australia, brains submitted for exclusion of transmissible spongiform encephalopathies (TSEs) as part of the National TSE Freedom Assurance Program must be whole and intact (Anon 2017). There are currently no tests to diagnose TSE in live animals, and so diagnosis requires necropsy and microscopic examination of the brains from ruminants that have shown signs of neurological disease.

Firearms or captive bolts are unsuitable methods of euthanasia for TSE surveillance because they cause extensive damage to the brain and brainstem. Consequently, veterinarians typically use an injectable barbiturate anaesthetic (eg pentobarbitone; PENT) in this situation, but this creates the risk of poisoning scavenging animals (including farm dogs and wildlife) due to the chemical residues in the carcase. Poisoning through the ingestion of barbiturate-laden carcases has caused the death of animals fed or scavenging carcase parts up to two years later (Kaiser *et al* 2010; Payne *et al* 2015). Consequently, barbiturates should only be used when a carcase can be disposed of immediately by deep burial or incineration (Leary *et al* 2013). These disposal requirements present difficulties for farmers due to cost and may discourage them from requesting disease investigations.

Euthanasia of ruminant livestock using saturated salt solutions, such as magnesium sulphate (MGS) or potassium chloride (KCL) provides a potential alternative to barbiturate use. These saturated salt solutions are not controlled substances, they are cheap, readily available, easily stored and transported, can be prepared in the field and pose minimal risk

of poisoning scavengers (Leary *et al* 2013). Since carcases containing saturated salt solutions are potentially less toxic to scavenging animals compared to barbiturates, deep burial of carcases may not be necessary (Leary *et al* 2013).

Further, saturated salt solutions have been used in the field by large animal veterinarians in Australia to euthanase animals following heavy sedation with an alpha-2 agonist, such as xylazine. Field observations indicated that the transition to death using MGS appeared smooth, occurring rapidly with no tetanic spasms, and with no other overt signs to indicate pain or distress. Field observations reported for KCL are rapid transition to death accompanied by tetanic spasms and involuntary muscular activity, sometimes violent. Xylazine has been the preferred sedative because of the deep sedation achieved at relatively low dose (Plumb 2018).

The current American Veterinary Medical Association (AVMA) euthanasia guidelines recommend that MGS and KCL should only be used to euthanase unconscious vertebrate animals (Leary *et al* 2013). High dose administration of saturated salt solutions are often associated with cardiac arrest because they exert an effect at a cellular level affecting cardiac, muscle and nerve function (Riviere & Papich 2018). Myocardial infarction in humans, commonly termed a heart attack, causes ischaemic tissue damage and pain (Menon *et al* 2019). Consequently, there is concern that cardiac arrest events that result from the administration of saturated salts may cause pain in a conscious animal. The assumption has been that deep sedation using an alpha-2 agonist (eg xylazine) followed by the administration of either KCL or MGS may not be humane methods of euthanasia, because the alpha-2 agonist does not induce unconsciousness (Dewell *et al* 2013; Shearer 2014). Xylazine can induce a state of sedation that resembles anaesthesia because it causes heavy sedation and provides some analgesia and muscle relaxation, but it does not render an animal completely unconscious (Evers *et al* 2006).

To date, there are no published studies that have directly evaluated the perception of pain or distress associated with euthanasia using MGS or KCL in ruminant livestock heavily sedated with xylazine. Anecdotal evidence reported by field veterinarians in Australia suggests that euthanasia with MGS after heavy sedation with xylazine can result in a rapid and smooth death. However, absence of observed adverse behaviours does not necessarily equate to the absence of perceived distress by an animal. Therefore, a scientific evaluation of the potential for distress associated with these methods was needed to determine whether they are humane and could be recommended for general use.

The measurement of brain activity by electroencephalography (EEG) has been used to evaluate cortical awareness and the perception of pain in animals, particularly in relation to killing methods for meat supply (Murrell & Johnson 2006; Gibson *et al* 2009). The validity of EEG to evaluate conscious perception of pain is well established for both humans and animals (Chang *et al* 2001; Murrell & Johnson 2006; Rault *et al* 2014). EEG can be used to assess nociception by the cerebral cortex, with painful stimuli causing a change in the components of frequency including F50, F95 and P_{tot}. These parameters are indirect measures of pain, but F50 and P_{tot}, in particular, are strongly correlated with noxious stimulation (Murrell & Johnson 2006; Gibson *et al* 2009). While F95 has also been correlated with nociception in some studies, the response is variable (Gibson *et al* 2009). However, changes in F95 are reliably associated with depth of anaesthesia. In the context of this study, F95 was included as an index of sedation following xylazine administration.

The objective of this study was to determine the effectiveness of KCL or MGS as euthanasia agents following heavy sedation with xylazine, using a sheep model. The movement and physiological responses and brain activity (EEG) of sheep were measured. Pentobarbitone was used for comparison.

Materials and methods

This study was approved by the Animal Ethics Committee of Massey University, New Zealand (MUAE 1811).

Study animals and treatment groups

Thirty-two, recently shorn, mixed age Romney cross ewes were utilised for this study. Animals were transported approximately 50 km from the saleyards to a Massey University research unit and housed as a single group in an outdoor paddock.

Sample size calculation was based on the assumption that nociception (F50) would be half that recorded during an experiment examining differences in dehorning with and without local anaesthetic (Gibson *et al* 2009). Sample size calculations (using alpha of 0.05 and beta 0.8) suggest a sample size of 20 (ten in each group) would detect a mean difference of 1.1 hz (control 3.6 hz and treatment 4.7 hz) with 94% confidence.

Animals were allocated to one of the three treatment groups using a random number generator and processed over five trial days. Treatment groups included euthanasia with either PENT, KCL or MGS. Stock solutions for KCL (KCl 97%, Dead Sea Works, Sdom, Israel) and MGS ($MgSO_4$ 95–100%, Ravensdown, Christchurch, New Zealand) were made based on their solubility at 30°C (Rumble 2018) with the supernatant decanted for intravenous administration. At 30°C, the saturation point for KCl and $MgSO_4$ is 37.2 and 39.7 g per 100 ml of water, respectively.

Euthanasia procedure

All animals were weighed (± 0.5 kg; XR5000, Tru-Test™, QLD, Australia) and their body condition score estimated (on a scale of 1 to 5; 1 being extremely thin and 5 being obese [Kenyon *et al* 2014]). Immediately before euthanasia, animals were heavily sedated with xylazine (0.4 mg kg^{-1} IM administered, registered dose range for ruminants: 0.01–0.3 mg kg^{-1}) (Kästner 2006).

Once recumbent, an indwelling jugular catheter and EEG electrodes were fitted. Subcutaneous 27-gauge, 0.5-inch stainless steel needle electrodes (Ambu, Ballerup, Denmark) were used to record the EEG bilaterally. A five-

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electrode montage was used with non-inverting electrodes positioned parallel to the midline between the medial canthi of the eyes, inverting electrodes positioned over the left and right mastoid processes and a common earth electrode located caudal to the poll on the left side of the neck (Mayhew & Washbourne 1990).

Once baseline data (at least 15 s of interpretable EEG trace) had been recorded from the sedated animal, the euthanasia agent was administered intravenously. Pentobarbitone (150 mg kg^{-1} IV, Pentobarb 500, Provet NZ Pty Ltd, New Zealand) was administered over a 60-s period as per label recommendations. For each saturated salt solution, 1,000 ml of supernatant was transferred into an IV fluid bag that was positioned approximately 850 mm above the catheter site to facilitate fluid flow by gravity. Infusion of KCL and MGS continued until cardiorespiratory arrest with permanent isoelectric EEG waveform activity was detected and involuntary reflexes were lost (absence of rhythmic breathing and audible heartbeat, absence of a blink reflex, dilated pupils, loss of jaw and tongue tone, absences of a corneal reflex).

Data collection

All euthanasia events were video-recorded from before infusion of the euthanasia agent until clinical death had occurred. Latency to the end of rhythmic breathing and inaudible heart rate were recorded as determined by continuous sampling thoracic auscultation by an experienced veterinarian. Both measures are crude estimates of clinical end-points because they can be affected by movement events.

The EEG signals were amplified using isolated differential signal amplifiers (Iso-Dam isolated biological amplifier, World Precision Instruments, Sarasota, FL, USA) and recorded with a gain of 1,000 and band-pass of 1.0–500 Hz. The EEG signal was digitised at a rate of 1 kHz (Powerlab 16/30, AD Instruments Ltd, Sydney, NSW, Australia) and analysed off-line at the conclusion of the experiment.

The EEG activity was recorded on three occasions: prior to the administration of any medication (baseline EEG), after xylazine had taken effect ('post-sedation EEG') and from the start of infusion until permanent isoelectric waveform could be confirmed in real time ('infusion EEG').

Latency to observed muscular activity (eg kicking, paddling) was also recorded. Time stamps and video recordings were retrospectively matched and compared with EEG traces to differentiate movement artefact associated with sedation (ie respiratory interference) from movement events that occurred during euthanasia. Movement events associated with euthanasia were classified using an ethogram developed for this study (Table 1), based on an ethogram used for pigs (Raj 1999).

Volume of saturated salt was recorded before and after infusion. The total volume of saturated salt solution required to cause death was calculated retrospectively based on the rate of infusion, volume infused and the time until permanent isoelectric EEG waveform was detected.

Table 1 The classification of movement events occurring in response to intravenous administration of either pentobarbitone or a saturated salt solution (KCL or MGS) to sheep, heavily sedated with xylazine (0.4 mg kg^{-1} IM). If the movement stopped for more than 2 s, any new movement displayed was considered a new movement event.

Classification	Duration	Movement
Mild	Brief: < 2 s	Muscle twitch
		Muscle contraction
		Head extension
		Spinal flexion
Moderate	Intermediate: 2–5 s	Kicking/paddling for < 5 s
Severe	Prolonged: > 5 s	Tetanic spasms/convulsions

EEG data analysis

All statistical analyses were performed using SAS 9.4 (SAS Institute Inc, Cary, NC, USA). Data were collected from both cerebral hemispheres, but only data from the right cerebral cortex were analysed because previous studies using a similar model have demonstrated equivalence in spectral EEG between hemispheres (Murrell & Johnson 2006).

Infusion EEG was compared with post-sedation EEG and subsequently classified and interpreted using methods described previously (Newhook & Blackmore 1982; Gibson *et al* 2009; Sutherland *et al* 2016). The raw EEG recorded during infusion (infusion EEG) of euthanasia agent was compared with that recorded after xylazine administration (post-sedation EEG) and was classified and interpreted using methods described previously (Newhook & Blackmore 1982; Gibson *et al* 2009; Sutherland *et al* 2016). Briefly, the EEG traces were visually inspected and assigned to one of five categories defined below based on the amplitude, frequency, or presence of characteristic frequency/amplitude patterns.

Based on these previous studies, EEG activity was categorised as: Normal (amplitude and frequency similar to that of baseline); Transitional (amplitude less than 50% of baseline EEG with marked change in frequency component); Low frequency high amplitude (LFHA) (increased low frequency activity with a concurrent increase in amplitude); Burst suppression (active or transitional EEG interspersed with periods of isoelectric EEG lasting for 0.5 s or more); and Isoelectric (a stable trace with amplitude less than 12.5% of baseline, consisting of background noise with little or no low-frequency component).

The total power (P_{tot}), median frequency (F50) and 95% spectral edge frequency (F95) were calculated for consecutive 1-s epochs, using purpose-written software (Spectral Analyser, CB Johnson, Massey University, Palmerston North, New Zealand 2002). Epochs containing data that were over-scale, under-scale or out of range, were excluded from analysis. Fast Fourier Transformation was applied to each epoch, yielding sequential power spectra with 1-Hz frequency bins.

Table 2 Mean latencies (s) to end of rhythmic breathing, end of detectable heart-beat, onset of transitional EEG and onset of permanent isoelectric EEG in sheep euthanased with either sodium pentobarbitone (PENT), potassium chloride (KCL) or magnesium sulphate (MGS).

Variable	Treatment	n	Mean (± SEM) time	df	*F*-value	Pr > F
End of rhythmic breathing (n = 30)	PENT	9	32 (± 1.5)[a]	2	43.73	< 0.0001
	KCL	11	16 (± 1.3)[b]			
	MGS	10	16 (± 1.4)[b]			
End of audible heart-beat (n = 27)	PENT	8	138 (± 7.9)[a]	2	56.75	< 0.0001
	KCL	9	24 (± 7.4)[b]			
	MGS	10	60 (± 7.1)[c]			
Transitional EEG (probable loss of consciousness; n = 29)	PENT	8	29 (± 4.6)	2	2.69	0.087
	KCL	11	53 (± 8.9)			
	MGS	10	43 (± 5.5)			
Permanent isolectric EEG (brain death; n = 29)	PENT	9	41 (± 13.3)[a]	2	26.22	< 0.0001
	KCL	10	165 (± 12.6)[b]			
	MGS	10	64 (± 12.6)[a]			

Means with different superscripts differ (adjusted $P < 0.00167$).

Statistical analysis

Paired *t*-tests were used to evaluate the effect of xylazine on baseline EEG. The effect of treatment on the latency to cessation of rhythmic breathing and detectable heart-beat, along with the appearance of transitional and isoelectric EEG waveforms, was evaluated using generalised linear models that included treatment and day as fixed effects and sheep as a random effect. Where a significant treatment effect was found, Tukey-Kramer *post hoc* tests were performed to identify group differences.

To test for evidence of nociception following administration of euthanasia agents, the mean F50, F95 and P_{tot} were calculated and compared for three consecutive non-overlapping 15-s periods (infusion EEG): immediately preceding the start of infusion (P1); from 1–15 s after start of infusion (P2); and from 16–30 s after start of infusion (P3). Beyond this period EEG was not suitable for spectral analysis, due to the appearance of transitional or isoelectric waveforms in some individuals. The presence of transitional, LFHA, burst suppression or isoelectric EEG is reported to indicate insensibility (Newhook & Blackmore 1982; Gibson *et al* 2009), therefore it is not likely that conscious perception of noxious stimuli could occur when these waveforms were present. A mixed model was used to compare mean F50, F95 and P_{tot} between periods. The model used a first-order autoregressive correlation structure and included treatment and day as fixed effects, sheep as a random effect and period as a repeated measure. Where significant interaction effects were identified, *post hoc* pair-wise comparisons were performed on the variables of interest (periods within treatment) and resultant *P*-values manually corrected for multiple comparisons.

Plots of standardised residuals versus predicted values were evaluated to test the assumption of normally distributed within-group errors. The distribution of residuals for all EEG variables were found to approximate a normal distribution and were therefore considered suitable for parametric analysis.

Results

A total of 31 animals were enrolled in the trial (PENT: n = 10; KCL: n = 11; MGS: n = 10). The mean (± SD) live weight of animals in the PENT, KCL and MGS groups were within 1 kg of each other (45.8 [± 4.4], 48.7 [± 6.0] and 47.7 [± 7.3] kg, respectively). The mean (± SD) body condition score for animals were within 0.3 BCSs in the PENT, KCL and MGS groups (2.5 [± 0.3], 2.5 [± 0.3] and 2.6 [± 0.3], respectively).

Data from 26 sheep were included for analysis of xylazine effect on baseline EEG, with data from five sheep excluded from this analysis due to excessive amounts of EEG artefact. Xylazine induced a significant reduction in F50 (59% reduction, mean [± SD] difference = 4.83 [± 4.12]; $t_{[25]} = 5.98$; $P < 0.001$) and F95 (12% reduction, mean difference = 3.17 [± 2.17]; $t_{[25]} = 7.43$; $P < 0.001$) between the baseline EEG and post-sedation EEG waveforms, but no significant change in P_{tot} (4% increase, mean difference = 0.61 [± 8.52], $t_{[25]} = 0.36$; $P = 0.7190$).

For all treatments, end of rhythmic breathing (ie respiratory arrest) occurred before cardiac arrest but took significantly longer to occur for animals in the PENT group compared with KCL and MGS (Table 2). Latency to inaudible heart-

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Table 3 Effect of euthanasia agent (n = 3; pentobarbital[1], potassium chloride[2] or magnesium sulphate[3]) and period (n = 3; 1: 15 s prior to infusion, 2: 0–15 s and 3: 16–30 s after start of infusion) on the mean median frequency (F50), 95% spectral edge frequency (F95) and total power (P_{tot}) of sheep EEG.

Variable	df	*F*-value	Pr > F	Treatment	Period	LSM (± SEM)	Comparison (period within treatment)	*t*-value	Pr > F[4]
F50	39	3.71	0.007	PENT	1	2.97 (± 0.58)	1 vs 2	–1.57	1.00
					2	3.61 (± 0.58)	1 vs 3	0.17	1.00
					3	2.88 (± 0.60)	2 vs 3	1.68	1.00
				KCL	1	3.60 (± 0.46)	1 vs 2	1.96	0.51
					2	2.97 (± 0.46)	1 vs 3	0.38	1.00
					3	3.40 (± 0.57)	2 vs 3	–0.92	1.00
				MGS	1	3.38 (± 0.52)	1 vs 2	–1.04	1.00
					2	3.75 (± 0.52)	1 vs 3	–2.86	0.06
					3	4.80 (± 0.54)	2 vs 3	–2.67	0.10
F95	39	3.29	0.021	PENT	1	24.43 (± 0.88)	1 vs 2	–0.81	1.00
					2	24.84 (± 0.88)	1 vs 3	1.61	1.00
					3	23.28 (± 0.90)	2 vs 3	2.86	0.06
				KCL	1	23.89 (± 0.70)	1 vs 2	0.72	1.00
					2	23.59 (± 0.70)	1 vs 3	–1.36	1.00
					3	24.82 (± 0.82)	2 vs 3	–2.09	0.39
				MGS	1	23.98 (± 0.78)	1 vs 2	–0.22	1.00
					2	24.08 (± 0.78)	1 vs 3	–0.34	1.00
					3	24.20 (± 0.81)	2 vs 3	–0.24	1.00
P_{tot}	39	2.94	0.032	PENT	1	14.89 (± 2.31)	1 vs 2	1.10	1.00
					2	13.02 (± 2.31)	1 vs 3	–0.36	1.00
					3	15.73 (± 2.40)	2 vs 3	–1.48	1.00
				KCL	1	16.20 (± 1.85)	1 vs 2	–1.44	1.00
					2	18.91 (± 1.85)	1 vs 3	0.84	1.00
					3	15.06 (± 2.31)	2 vs 3	1.98	0.49
				MGS	1	20.20 (± 2.06)	1 vs 2	0.51	1.00
					2	20.23 (± 2.06)	1 vs 3	2.30	0.24
					3	16.19 (± 2.18)	2 vs 3	2.31	0.24

[1] PENT; [2] KCL; [3] MGS; [4] Corrected *P*-values = raw *P*-value multiplied by nine comparisons of interest (adjusted $P < 0.0056$).

beat (ie cardiac arrest) occurred significantly quicker for animals in the KCL group compared with MGS and PENT. Latency to inaudible heart-beat occurred significantly quicker for animals in the MGS group compared with PENT.

The time to transitional EEG (ie probable loss of consciousness) was comparable across all treatment groups. The onset of permanent isoelectric EEG (ie brain death) occurred significantly quicker for animals in the MGS and PENT groups compared with the KCL group.

There was no evidence of nociception following administration of any euthanasia treatment, as measured by changes in F50, F95 and P_{tot}, prior to the onset of EEG waveforms considered incompatible with conscious awareness. Although a significant Period × Treatment effect was identified for each EEG measure (F50: $P = 0.007$; F95: $P = 0.021$; P_{tot}: $P = 0.032$), *post hoc* pair-wise comparisons between periods within treatment, revealed no significant interaction effects for F50, F95 or P_{tot} (Table 3).

Table 4 A summary of the number of movement events in sheep undergoing euthanasia with either sodium pentobarbital, saturated potassium chloride solution or saturated magnesium sulphate solution.

	Number of movement events (%)		
	Mild	**Moderate**	**Severe**
Pentobarbital	1 (100)	0 (0)	0 (0)
Saturated potassium chloride solution	23 (48)	12 (25)	13 (27)
Saturated magnesium sulphate solution	6 (75)	2 (25)	0 (0)

Mild response: < 2-s duration, movements included muscle twitch, muscle contraction, head extension, spinal flexion; Moderate response: 2–5-s duration, include mild movements of longer duration, or kicking or paddling for < 5 s;
Severe response: > 5-s duration, included sustained moderate responses and tetanic spasms/convulsions.

Data from 27 sheep were included in the analysis of nociception associated with euthanasia (PENT: n = 7; KCL: n = 11; MGS: n = 9). Data from four sheep (PENT: n = 3; MGS: n = 1) were excluded due to the presence of extensive movement artefact in all three periods (Table 4). These movement artefacts were associated with increased respiratory effort that developed after xylazine was administered and were not related to administration of PENT or MGS.

The results of EEG classification for all individuals, grouped by treatment, are illustrated in Figure 1. Where movement artefact occurred (cross-hatched bars), it was not possible to classify the EEG. Periods of normal EEG indicate compatibility with conscious awareness (black bars). Transitional EEG represents a state that is probably not compatible with awareness (grey bars), whereas the occurrence of burst suppression (black lines), low-frequency-high-amplitude (light grey bars), or isoelectric EEG (white bars) are considered incompatible with conscious awareness (Newhook & Blackmore 1982; Gibson *et al* 2009; Sutherland *et al* 2016).

More than 90% (57/62) of movement artefacts identified (cross-hatched bars; Figure 1) were associated with movement events as identified by review of video recordings (PENT, n = 1; KCL, n = 48; MGS, n = 8). Animals treated with KCL demonstrated the most frequent movement events per animal (4.3 [± 1.4] events). Nine of the eleven animals in the KCL groups exhibited severe movement events during euthanasia that included sustained kicking, paddling, spinal flexion and/or tetanic convulsions. These movement events did not appear to be associated with nociception because animals were unconscious before and after the events. No severe movement events were noted in the MGS or PENT groups. There were few movement events noted for the MGS group and a majority of these were classified as mild (six of eight; Table 4).

The required volume of saturated salt solution to reach the onset of permanent isoelectric EEG waveform was 150 ml (range: 39–358 ml) and 50 ml (range: 38–68 ml) for KCL and MGS, respectively. This equated to approximately 1–7 ml kg^{-1} and 1–2 ml kg^{-1} live weight for KCL and MGS, respectively.

Discussion

Based on EEG assessment and timing and type of movements observed, this study provides evidence that the administration of saturated salt solutions for euthanasia of sheep that are heavily sedated with xylazine is humane. This method of euthanasia is useful when captive bolts, firearms or pentobarbitone cannot or should not be used.

Previous authors have questioned if this method of euthanasia is humane because xylazine does not induce a state of unconsciousness and thus animals may perceive pain or may experience distress (Leary *et al* 2013; Shearer 2014). Results from the present study demonstrate that although xylazine did not induce a state of unconsciousness, animals did not consciously perceive pain associated with the administration of KCL or MGS when compared with the PENT group based on EEG assessment.

Changes in the frequency spectrum of the EEG were used to assess level of sedation associated with xylazine administration and nociception associated with infusion of saturated salt solutions. Previous studies have demonstrated that increasing depth of sedation or anaesthesia results in a shift from low amplitude, high frequency EEG activity (seen in awake or very lightly anaesthetised mammals) to a high amplitude, low frequency waveform (Otto & Short 1991; Johnson 1996; Antunes *et al* 2003). Such a shift is associated with reductions in F95 and F50. While reductions in F95 are consistently observed with increased agent concentration, irrespective of agent, changes in F50 are more variable (Johnson 1996; Johnson *et al* 1999), leading to the conclusion that F95 is a specific marker of central nervous system suppression, whereas F50 is specific to anti-nociception (Johnson *et al* 1999). Deeper anaesthesia is characterised by the appearance of burst suppression, characterised as periods of active EEG interspersed with isoelectric bursts of ≥ 1 s and eventual isoelectric EEG (amplitude approximately less than 10% of baseline waveform). Before the appearance of isoelectric EEG, a reduction in the amplitude of the raw EEG may become evident. Transitional EEG is considered representative of likely loss of awareness, whilst isoelectric EEG is considered incompatible with awareness (Newhook & Blackmore 1982; Gibson *et al* 2009; Sutherland *et al* 2016). As such, the permanent appearance of either waveform is considered indicative of loss of awareness, or an inability to perceive by the senses.

In the context of xylazine administration, the aim was to determine whether the prescribed dose was associated with sedation (as assessed by a reduction in F95) and/or changes in awareness (as assessed by a ≥ 50% reduction in EEG amplitude). In the present study, the decrease in the 95% spectral edge frequency of the EEG following xylazine administration is consistent with that seen with increasing depth of inhalant anaesthesia (Otto & Short 1991; Johnson 1996; Antunes *et al* 2003), indicating heavy sedation. The reduction in F50 observed following xylazine administration is consistent with results previously observed after the administration of anti-nociceptive

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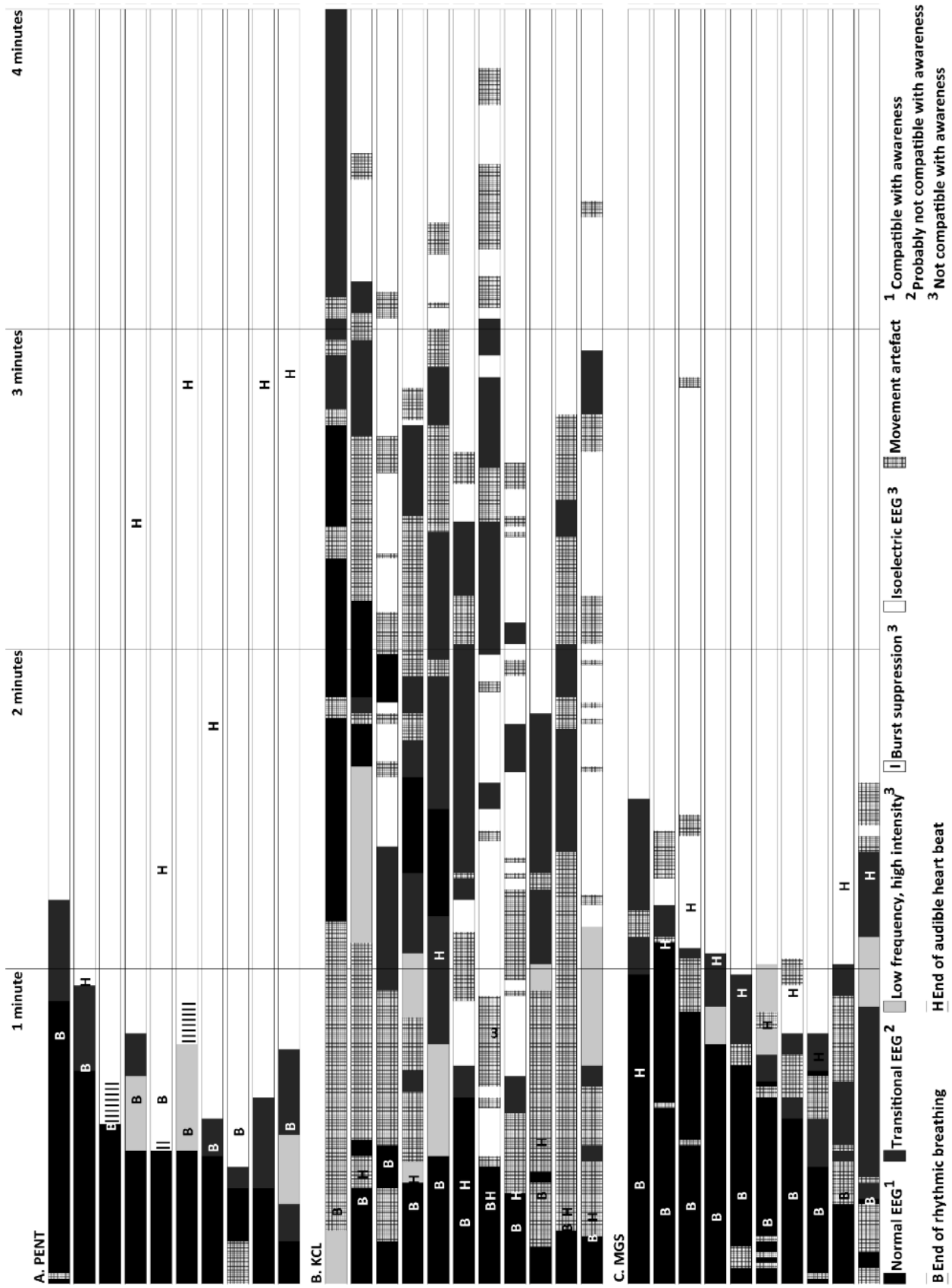
Figure 1



Electroencephalography (EEG) activity of sheep that were euthanased via intravenous infusion of either pentobarbitone (a), saturated KCL (b) or saturated MGS (c) following heavy sedation with xylazine (0.4 mg kg^{-1} IM).

drugs (Johnson *et al* 1999) indicating that xylazine has an analgesic effect at the dose provided.

Previous studies using EEG to evaluate animal responses to noxious stimuli under light halothane anaesthesia have demonstrated that nociception is reflected by a significant and transient (60–90 s) increase in F50 and F95, and a corresponding decrease in P_{tot} (Murrell & Johnson 2006; Gibson *et al* 2009). Similarly, an increase in the mid-frequency component of the EEG was reported in conscious sheep subjected to noxious electrical stimuli (Ong *et al* 1997). In the present study, no such changes in EEG frequency components were observed in response to any of the three euthanasia agents. This demonstrates that in sheep that were heavily sedated with xylazine, there was no conscious perception of pain associated with the infusion of either PENT, KCL or MGS, or the resultant cardiorespiratory arrest events. The appearance of transitional or isoelectric EEG waveforms beyond this time-point prohibited further comparison of frequency components, but nociceptive inputs beyond this point would not be consciously perceived.

It was not possible to determine if this finding was associated with the analgesic or anti-nociceptive properties of xylazine (Riviere & Papich 2018) or whether the infusion of the saturated salt solutions did not evoke a nociceptive response. Further studies could examine the anti-nociceptive properties of xylazine in sheep using a minimal anaesthetic model (Murrell & Johnson 2006).

Although the sheep did not perceive pain associated with administration of KCL or MGS, clinical endpoint and movement data support the preferential use of MGS over KCL. Time until the probable loss of consciousness (transitional EEG) was comparable for KCL and MGS, but sheep euthanased with KCL consistently exhibited severe movement events during infusion. These movement events commonly included violent kicking, leg paddling and sustained spinal flexion or convulsion. These movement events typically began shortly after the start of infusion and continued during and after transitional and isoelectric EEG waveforms were observed. Because these movement events continued after loss of consciousness, they were deemed reflexive, rather than conscious responses and had no impact on the animals' welfare. Similar reflex movement including sustained kicking and paddling are common in livestock that have been rendered unconscious prior to slaughter at abattoirs (Verhoeven *et al* 2015).

Although these reflex movements do not impact the animals' welfare, with increasing scrutiny and awareness of animal welfare practices, such visually unappealing responses should be avoided where superior methods of euthanasia are available. Additionally, violent and unpredictable muscle spasms could pose a serious threat to the safety of personnel administering the KCL, particularly if large animals, such as cattle or horses, are being euthanased with this method. No severe movement events were observed in the MGS group.

The disparity in clinical endpoint and movement events between KCL and MGS are likely associated with their modes of action. Potassium chloride affects the resting membrane potential of cells and causes excitation, whereas MGS has neuromuscular blocking affect causing relaxation and central nervous system depression (Grimm *et al* 2015; Riviere & Papich 2018). Although no differences in the humaneness of KCL or MGS were evident, the difference in movement events alone are significant enough to support the preferential use of MGS.

Given the rapid and smooth transition to the onset of brain death observed in sheep euthanased with MGS in this trial, similar results could be expected if this method was used for other ruminant (ie cattle, goats) and non-ruminant livestock (ie horses, pigs). Ruminant livestock are extremely sensitive to the sedative effects of xylazine and require much lower doses to achieve heavy sedation compared with other mammals, including horses (Riviere & Papich 2018). Consequently, if this method of euthanasia was extrapolated for use in non-ruminant animals, the xylazine dose would need to be adjusted or combined with other drugs to achieve sternal recumbency.

In this study, larger volumes of KCL compared with MGS were required before permanent isoelectric waveform was observed. This result was unexpected because previous field observations reported that larger volumes of MGS were needed to euthanase an animal compared with KCL. This difference may be associated with the retrospective method of calculation used to determine the volume of saturated salt solution required to reach isoelectric endpoint, rather than the observation of clinical death in a field setting. As such, the recommendation of in-field use is that infusion with saturated salt solution continues until clinical death is confirmed. In this trial, 1–2 ml kg^{-1} live weight of MGS was required to euthanase a sheep, but the authors recommend preparing a minimum of 10 ml kg^{-1} live weight of MGS prior to the start of infusion.

Although residues in carcases were not specifically measured in this study, the risk of secondary poisoning associated with consumption of carcases containing KCL or MGS is deemed negligible. The AVMA guidelines currently recognise that one of the advantages of KCL and MGS is that the risk of secondary poisoning is reduced and when used in accordance with their recommendations, may be a good choice of euthanasia where carcase disposal is not possible (Leary *et al* 2013).

The risk of secondary poisoning associated with high doses of xylazine are also deemed negligible. Previous studies have reported that there were no treatment-related adverse effects in Beagle dogs that were fed 0.3 to 3 mg kg^{-1} per day for 1 to 13 weeks (Chamberlain & Brynes 1998). Based on these studies, a 20-kg dog could safely consume 60 mg of xylazine each day for 13 weeks with no adverse response. At the dose rates used in the present study, a 20-kg dog could consume three 50-kg sheep each day with no risk of

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toxicosis. The risk of oral toxicity for other birds or carnivorous wildlife species is not currently known. Avian species tolerate relatively high doses of xylazine (1–2 mg IM) with only light sedative effects (Riviere & Papich 2018) so large quantities of contaminated carcase would need to be consumed for serious effect, but the potential effects on other carnivorous species warrants further investigation.

There are few limiting factors to adoption of xylazine and saturated salt solutions for euthanasia. Xylazine is relatively cheap and commonly carried by farm animal veterinarians, and saturated salt solutions can be prepared in the field (Leary *et al* 2013). The salts are cheap, readily available and easily stored and transported. The relatively large volumes of saturated salt solutions (up to 1 L) required to euthanase cattle and horses may be seen as a disadvantage by some. The overwhelming advantage is the negligible risk of harming animals that might scavenge the carcase.

Animal welfare implications and conclusion

In situations where whole, intact brains are required, or when captive bolt or firearms are not available, or when carcase disposal is problematic, the use of saturated salt solutions in heavily sedated ruminant livestock provides a rapid, safe, humane and practical means of euthanasia. This study provides clear evidence that intravenous infusion of MGS or KCL, following deep sedation with xylazine, are humane methods of euthanasia of ruminant livestock. There was no evidence of perception of pain associated with the administration of either agent and consciousness was lost quickly. However, MGS is preferred because animals euthanased with KCL consistently exhibited violent, post mortem reflex movements that are visually unappealing and may compromise operator safety.

The intravenous infusion of MGS to heavily sedated animals should be continued until death has been confirmed. This method poses minimal risk to scavenging animals by secondary poisoning and requires no specialised equipment.

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