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Running head: Euthanasia of goat kids

Evaluation of the efficacy of a non-penetrating captive bolt to euthanise neonatal goats up to 48 hours of age

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Abstract

Manual blunt force trauma is a common method of euthanasia or culling of goat kids, however it is difficult to apply consistently and may vary in effectiveness. Therefore, a controlled mechanical method is needed. The overall objective of this research was to evaluate the effectiveness of a non-penetrating captive bolt (NPCB) to euthanize goats up to 48 h of age. In a pilot study (n = 27), the optimum anatomical site for placement of the NPCB was evaluated using signs of insensibility and death, and post-mortem assessment of traumatic brain injury. Three different anatomical sites (frontal bone, poll or behind the poll) were evaluated. In experiment 1 (n=100), goats were euthanized using the optimum anatomical placement determined in the pilot study and the presence of brainstem reflexes, rhythmic respiration, convulsions and cardiac activity were recorded. In experiment 2 (n = 7), electroencephalogram (EEG) was recorded to assess awareness following application of the NPCB. Results from the pilot study showed that immediate insensibility followed by death was achieved when the muzzle of the NPCB was positioned behind the poll and the goat's head was bent so that the chin touched the chest. In experiment 1, all goats were rendered immediately insensible without return to sensibility prior to cessation of cardiac activity. In experiment 2, application of the NPCB resulted in the immediate onset of EEG activity which was incompatible with awareness. In conclusion, the NPCB reliably caused immediate, sustained insensibility followed by death in goats up to 48 h of age.

Keywords: animal welfare, electroencephalogram, euthanasia, goats, insensibility, non-penetrating captive bolt

Introduction

Manually applied blunt force trauma (BFT) is a common method of euthanasia and culling for many neonatal species, including goat kids. However, manually applied BFT is difficult to apply consistently, is often aesthetically unpleasant for operators to perform and poses a significant public perception concern. In contrast, mechanically applied BFT performed using a penetrating (PCB) or non-penetrating captive bolt (NPCB) can deliver an appropriate and uniform amount of force resulting in more consistent structural damage to the brain (AVMA 2013). Therefore, industry and farm operators have recognised that there is a need to evaluate mechanical methods of BFT that cause immediate insensibility and death with minimal pain and distress to the animal.

Finnie *et al* (2000) found that a NPCB produced sufficient traumatic brain injury to suggest that it is an acceptable method of euthanasia for 4- to 5-wk-old lambs. Similarly, a NPCB device was found to be effective for euthanising pigs less than 3 d of age (Casey-Trott *et al* 2013), pigs weighing 3-9 kg (Casey-Trott *et al* 2014) and turkeys (Erasmus *et al* 2010a, b). However, little is known regarding the effectiveness of a NPCB as a method of euthanasia for goats up to 48 h of age.

When using mechanical methods of BFT to euthanise animals, correct anatomical placement is critical to ensure that adequate damage occurs to vital structures of the brain in order to cause immediate and sustained insensibility and death. In a study of PCB euthanasia of 489 sheep, 6% of animals showed signs of incomplete concussion, all of which were associated with inaccuracy of the shot upon post-mortem examination and the bolt missed the brain entirely in 79% of these animals (Gibson *et al* 2012). For euthanasia of neonatal goats, American Veterinary Medical Association (AVMA 2013) recommends that the PCB be placed on the intersection of two lines going from the lateral canthus of the eye to the horn on the opposite side. According to the World Organisation for Animal Heath Terrestrial Animal

Health Code (OIE 2015) the optimum placement for hornless sheep and goats is the highest point of the head, on the midline, with the captive bolt aimed towards the angle of the jaw. Alternatively, the Humane Slaughter Association (HSA 2008) recommends that the PCB be placed behind the midline and aimed towards the base of the tongue. However, little is known about the optimal anatomical placement of a NPCB when euthanising neonatal goats.

The objectives of this study were (1) to develop a protocol for NPCB euthanasia of neonatal goats, including the optimum anatomical site for placement of the NPCB muzzle, and (2) evaluate the effectiveness of the NPCB euthanasia protocol in regards to immediate and sustained insensibility and death when applied to goats up to 48 h of age.

Materials and methods

All procedures involving animals were approved by the AgResearch Ruakura Animal Ethics Committees under the New Zealand Animal Welfare Act 1999. The study was conducted between July and August (southern hemisphere winter) 2014 on commercial farms and performed on animals that farmers identified as needing to be euthanised. A pilot study, conducted in two parts, was first performed to aid in the design of the proceeding experimental work.

Euthanasia Device

A cordless, propane powdered NPCB (TED, BOCK Industries, Inc., Philipsburg, PA, USA) was used to euthanise all goats in the study. The mass of the NPCB bolt was 61.4 g and was released at a velocity of 30.1 m/s. The resulting energy produced by the NPCB bolt was 27.8 Joules (R. Bock, personal communication).

Pilot study

The aim of the pilot study was to evaluate the optimum anatomical site for placement of the NPCB muzzle; first by assessing the effect of placement of the NPCB on traumatic brain injury in anaesthetised goats and second, by assessing the effect of placement of the NPCB on signs of insensibility and death.

In part 1, fifteen (female, n = 3; male, n = 12) Saanan goat kids (4.0 kg ± 0.47 SD), less than 48 h of age, were allocated to one of three treatments (n = 5 / treatment; Figure 1): 1) the muzzle of the NPCB was placed on the frontal bone, at the intersection of two lines drawn from the lateral canthus of the eye to the region of the horn bud on the opposite side of the head, and with the lower jaw resting flat on a firm surface (FRONT), 2) the muzzle of the NPCB was placed on the top of the head (poll) with the lower jaw resting flat on a firm surface (POLL) or 3) the muzzle of the NPCB was placed behind the poll between the ears with the lower jaw resting flat on a firm surface (BACK). These anatomical landmarks were based on published recommendations for placement of a PCB for small ruminants according to the AVMA (2013), OIE Terrestrial Animal Health Code (OIE 2015) and HSA (2008) for the FRONT, POLL and BACK locations, respectively.

Goats were weighed, anaesthetised by administering 0.1 mL 2% Xylaxine (Phoenix Pharm Distributors Limited, Auckland, NZ) intramuscularly. Once anesthesia was confirmed, goats were individually placed in a purpose built portable rigid plastic restraint device, which had four holes in which to place the legs through and a firm surface to support the head. The restraint device was elevated so the legs of the animals hung down without touching the ground. The muzzle of the NPCB was positioned according to the allocated treatment and a single shot was fired. Five min after application of the NPCB, any goats that still had a heartbeat received an intravenous overdose of pentobarbitone (5 mL; Provet NZ Pty Ltd, Auckland, NZ), to ensure death.

Following death, goats were dissected by a trained veterinary pathologist blind to the treatments who scored the number of skull fractures and extent of brain haemorrhaging caused by application of the NPCB. The skin was removed from the dorsal surface of the skull and the amount of haemorrhaging in the subcutaneous tissue was assessed. If fracture lines were visible in the skull they were counted. The head was then disarticulated from the spinal column at the occipitoatlantal joint and cut longitudinally in the midline on a band saw. The extent of intracranial haemorrhage was assessed on the dorsal surface of the brain. The brain was then dissected out and the floor of the cranial cavity was examined for fractures. Haemorrhages underneath the scalp on the dorsal surface of the skull, in the area over the occipital bone, were classified as subcutaneous (SC) haemorrhages. Haemorrhages on the dorsal surface of the brain, after the skull and dura were removed, were classified as subdural dorsal (SDD) haemorrhages. Both SC and SDD haemorrhages were scored based on the macroscopic scoring system described in Casey-Trott *et al* (2013): 1: no haemorrhages present, 2: haemorrhages 0 to 2 cm in diameter, 3: haemorrhages 2 to 4 cm in diameter and 4: haemorrhages > 4cm in diameter.

In part 2, 12 (female, n = 4; male, n = 8) Saanan goat kids (4.2 kg ± 0.66 SD), less than 48 h of age, were allocated to one of four treatments (n = 3 / treatment; Figure 1): FRONT, POLL, BACK and BACK-MOD. The NPCB was applied in the same way for FRONT, POLL, and BACK as described in part 1 of the pilot study. The BACK-MOD treatment involved placing the NPCB behind the poll between the ears with the goats head bent so its chin was touching its chest. The purpose of this treatment was to focus the force of the NPCB closer to the thalamus, midbrain and pons regions of the brain.

Goats were weighed then placed individually in the restraint device. The muzzle of the NPCB was positioned according to the allocated treatment and a single shot was fired. Treatments were performed in a random order. Immediately after application of the NPCB and every 30 seconds thereafter, signs of sensibility (presence of brainstem reflexes) were assessed together with presence of cardiac activity, convulsions and rhythmic respiration, until cardiac activity ceased or for a maximum period of 15 minutes. After application of the NPCB, if any animal showed signs of sensibility (presence of brainstem reflexes or rhythmic respiration) at any stage the NPCB was applied a second time to ensure insensibility followed by death. Brainstem reflexes measured included corneal reflex and response to a painful stimulus. The corneal reflex involved touching the surface of the eye to provoke an eye blink response. To assess the response to a painful stimulus, a needle prick was applied to the nose to provoke a withdrawal response. The presence of rhythmic respiration and convulsions were monitored visually and the presence of cardiac activity was determined by palpation. Convulsions were defined as the total of clonic and tonic neuromuscular leg spasms (e.g. leg paddling and rigid leg extensions).

Experiment 1

One hundred (female, n = 16; male, n = 84) Saanan goat kids (3.9 kg ± 0.60 SD), less than 48 h of age, were used to evaluate the efficacy of the NPCB. Goat kids were sourced from four commercial farms. Goat kids were euthanised on-farm by five stock people, who were routinely responsible for euthanising animals' on-farm and who were trained in the use of the NPCB and two science technicians who were trained to use the NPCB. Farm 1 had two stock people and farms 2 and 4 had one stock person per farm involved in the study. All experimental animals were euthanised by the trained technicians on farm 3 and the technicians euthanised 50 – 67% of the goats from farms 1, 2 and 4.

Goats were weighed then placed individually in the restraint device. The BACK-MOD placement for the NPCB was used. This anatomical placement was chosen as it caused immediate insensibility and death in goats in the pilot study. A single shot was fired.

Assessment of insensibility and death was the same as described in part 2 of the pilot study. After application of the NPCB, if any animal showed signs of sensibly at any stage the NPCB was applied a second time to ensure insensibility followed by death. If cardiac activity was still present after 15 min then an overdose of Xylazine (2% Xylaxine; Phoenix Pharm Distributors Limited, Auckland, NZ) was administered intramuscularly to ensure death. The first 10 goats euthanised in this study were collected and assessed for traumatic head injury using the same post-mortem methodology described in part 1 of the pilot study.

Experiment 2

Seven male Saanan goat kids, less than 48 h of age, were used to evaluate the effect of the NPCB on latency to loss of awareness.

Anaesthesia

An established minimal anaesthesia model was adapted for use in the goat. Anaesthesia was induced in goat kids using 4% halothane (Halothane-Vet; Merial NZ Limited, Manukau City, NZ) vaporised in oxygen (4 L/min). Once an adequate depth of anaesthesia had been achieved, confirmed by visual inspection, halothane delivery was adjusted to maintain an end-tidal tension of 0.95–1.05 % for the remainder of anaesthesia period. End tidal halothane and CO_2 tension were monitored throughout using an anaesthetic agent monitor (Hewlett Packard M1025B, Hewlett Packard, Hamburg, Germany). Rectal temperature was monitored using a digital thermometer (Q 1437; Dick Smith Electronics, NZ) and body temperature maintained with the aid of a heating pad (T pump; Gaymar Industries Inc., Orchard Park, NY, USA).

EEG recording

Twenty-seven gauge stainless steel needle electrodes (Viasys Healthcare, Surrey, England) were positioned subcutaneously to record EEG and electrocardiograph (ECG) activity. A five-electrode montage was used to record EEG from both the left and right cerebral hemispheres, with non-inverting electrodes placed parallel to the midline over the left and right frontal bone zygomatic processes, inverting electrodes over the left and right mastoid processes and a ground electrode placed caudal to the occipital process (Murrell & Johnson 2006). Electrocardiograph was recorded using a base-apex configuration.

Both EEG and ECG signals were amplified with a gain of 1000 and a band-pass of 1.0–500Hz (Iso-Dam isolated biological amplifier, World Precision Instruments Inc., Sarasota, FL, USA) and digitised at a rate of 1 kHz (Powerlab 4/20, ADInstruments Ltd, Colorado Springs, CO, USA). Once end tidal halothane tension was stable at 0.95–1.05 %, EEG was recorded for 5 min prior to application of the NPCB (baseline) and for a further 10 min after application. The NPCB was positioned according to the description in experiment 1, and a single shot was fired. The digitised signals were recorded on an Apple Macintosh personal computer for off-line analysis at the conclusion of the experiment.

The EEG recorded following the application of the NPCB was compared to the baseline values and in a similar manner to Gibson *et al* (2009). Four categories were identified: 1) Normal: amplitude and frequency similar to baseline period; 2) Epileptiform: amplitude increased with increased low frequency activity, 3) Transitional: amplitude less than 50% of baseline and 4) Isoelectric: Amplitude less than 12.5% of baseline.

Statistical Analysis

Data from the pilot study and experiment 2 are descriptive only and are presented as means and ranges in the table and figures. Data from experiment 1 were tested for homogeneity of variance and normal distribution then subjected to analysis of variance using the mixed model procedure of SAS version 9.3 (SAS Inst., Inc., Cary, NC, USA). The mixed model was used to test for overall mean differences among operators or goat gender on time to cessation of cardiac activity and convulsions. Farm was included as a random effect. Statistical significance was determined at $P \leq 0.05$ and $0.05 < P \leq 0.10$ were considered a tendency.

Results

Pilot study

In part 1, the number of skull fractures and the haemorrhage scores are presented in Table 1. More skull fractures and higher SC haemorrhage scores were observed at the front of the skull and in the region of the frontal and parietal lobes of the brain when the NPCB was placed on the FRONT or POLL. Conversely, more skull fractures and a higher SC haemorrhage scores were observed at the back of the skull and in the region of the occipital lobe of the brain when the NPCB was placed on the BACK. However, the degree of SDD haemorrhaging was similar in the region of the frontal and parietal lobes of the brain among all three anatomical placements of the NPCB. From these preliminary results it was not evident which anatomical placement would most reliably cause immediate and sustained insensibility and death in goats, therefore the effect of anatomical placement of the NPCB on signs of insensibility and death was assessed in part 2 in conscious goats.

In part 2, immediate insensibility was not achieved in any of the three FRONT goats; all three goats exhibited rhythmic respiration and brainstem stem reflexes were also present in one goat after application of the NPCB. After applying the NPCB to the POLL, the first animal became immediately insensible and remained insensible until cessation of cardiac activity, but rhythmic respiration continued in the following two goats after application of the NPCB. Application of the NPCB to the BACK resulted in the first goat becoming immediately insensibility, however the corneal reflex and rhythmic respiration returned 2.5

min after triggering the device. The other two BACK goats became immediately insensible and remained that way until death. Due to the variable results achieved placing the NPCB at the FRONT, POLL or BACK it was decided to position the head so that the goats chin was touching its chest, to more reliably focus the force of the NPCB closer to the thalamus, midbrain and pons regions of the brain. All three BACK-MOD goats became immediately insensible and remained insensible until cessation of cardiac activity.

Experiment 1

All goats were rendered immediately insensible and no animals showed signs of returning to sensibility prior to death. Only one out of 100 goats needed to be euthanised using an alternative method as cardiac activity had not ceased within 15 minutes; this animal showed no signs of returning to sensibility within that time. The average time to cessation of cardiac activity and convulsions are presented in Table 2. The cumulative percentage of goats ceasing cardiac activity and convulsion was categorized into 1-min intervals and plotted across time (Figure 2).

More skull fractures and a higher SC haemorrhage score was observed at the back of the skull and in the region of the occipital lobe of the brain when the NPCB was placed on the BACK-MOD compared with FRONT and POLL (Table 1). More SDD haemorrhaging occurred around the caudal end of the brain, closer to the vital centres of the brainstem.

There was no effect of operator on the time to cessation of cardiac activity ($P = 0.608$) or convulsions ($P = 0.807$; Table 2). In addition, there was no effect of goat gender on the time to cessation of cardiac activity ($P = 0.146$) or convulsions ($P = 0.819$; Table 2).

Experiment 2

Apnoea, as judged by the absence of carbon dioxide in the respiratory gas sample, developed immediately following application of the NPCB and persisted for the duration of the recording period in all animals. Changes seen in the EEG following application of the NPCB are illustrated in Figure 3. After a period of movement artefact lasting between 0.7 and 4.2 s (mean 2.1 s), EEG was found to be either epileptiform or isoelectric (definitely not compatible with awareness) in three animals and transitional (probably not compatible with awareness) in the other four animals. The first instance of either epileptiform or isoelectric EEG was seen in all animals between 0.7 and 27.1 s (mean 7.5 s) after application of the NPCB. No periods of normal EEG activity were seen in any animals at any time following application of the NPCB. Changes seen in the EEG for the first 5 min following application of the NPCB are illustrated in Figure 3.

Discussion

The word euthanasia is derived from the Greek terms 'eu' and 'thanatos' which combined mean good death. Therefore, the term euthanasia refers to ending an individual animal's life with minimal pain or distress (AVMA 2013). The NPCB caused immediate and sustained insensibility until death, hence a humane death in goat kids up to 48 h of age. However, correct anatomical placement of the NPCB and positioning of the head were important characteristics related to the efficacy of this device. Recommendations regarding the optimal anatomical placement when using a PCB to stun or kill neonatal ruminants (calves, lamb and kids) are available (HSA 2008; AVMA 2013; OIE 2015), however these recommendations are based on research using different PCB/NPCB devices and few studies have investigated the optimum anatomical placement of a NPCB (in particular the TED used in this study) to euthanise goats up to 48 h of age.

All 100 goats (experiment 1) in the present study were rendered immediately insensible and remained insensible until death, confirmed using cessation of cardiac activity, when the NPCB was applied behind the poll with the animals chin touching its chest. These results are similar to Casey-Trott *et al* (2013) who found that a NPCB caused immediate insensibility in 100 neonatal pigs less than 3 d of age. These results suggest that applying the NPCB to the back of the kids head (between the ears) with the head bent over is an effective method of euthanasia for goat kids up to 48 h of age.

In the present study, the extent of traumatic brain injury caused by application of the NPCB to different anatomical sites using sedated animals was initially investigated. Applying the NPCB to the FRONT or POLL of the head caused more haemorrhaging around the frontal region of the brain compared to placing the NPCB behind the midline which caused more damage to the occipital region of the brain, near the thalamus and brainstem. Casey-Trott *et al* (2013) also found that a NPBC caused severe brain haemorrhaging in pigs less than 3 d of age, resulting in significant damage to the brainstem, cortex and subcortical tissues. These parts of the brain are responsible for vital life functions such as breathing and therefore should be the areas targeted when using a NPCB. Evaluation of traumatic brain injury alone was not sufficient to confirm the optimum anatomical placement of the NPCB to cause immediate and sustained insensibility in goats, therefore the NPCB was applied to the same three anatomical sites but using conscious animals. It was quickly evident that applying the NPCB to the FRONT, POLL or BACK locations was not effective, however placing the NPCB behind the midline with the goats head bent was. Correspondingly, Gibson *et al* (2012) was able to successfully euthanise adult sheep with a PCB placed on the midline of the head (similarly to the FRONT treatment in the current study). However, it is likely that the devices used by Gibson *et al* (2012) caused more overall structural brain damage than the device used in the present study since they were penetrating and/or fired using higher calibre cartridge charges.

Gibson *et al* (2012) also demonstrated the importance of correct placement of the captive bolt to ensure that animals become insensible immediately and remain in that state until death. In addition, placement of a PCB was found to be an important factor in causing sufficient disruption to the brainstem in cattle (Gilliam *et al* 2012). The results from the current study highlight the importance of evaluating different captive bolt devices on the species and age group on which they are attended to be used on as it is likely that anatomical placement of devices and appropriate head position will vary based on the type and power of the device.

Cessation of cardiac activity is often used to confirm death. The average duration of cardiac activity was 8.2 min in goats less than 48 h of age in the present study. Similar values were reported for neonatal pigs euthanised with an NPCB; cardiac activity ceased in pigs less than 3 d of age at 7.0 min (Casey-Trott *et al* 2013) and at 8.7 min in pigs between 3 - 9 kg (Casey-Trott *et al* 2014). In adult polled ewes, however, cessation of cardiac activity upon application of a PCB appeared to be more variable (0.25 - 13.7 min; Gibson *et al* 2012). Continuation of cardiac activity after brain death is due to stimulation from the autonomic nervous system independent of cerebral regulation (Cooper *et al* 1989). Therefore, although one goat (1/100 goats) was euthanised using a secondary method due to continuation of cardiac activity up to 15 minutes after application of the NPCB, it is likely that this animal was brain dead as during this period brainstem reflexes were absent including rhythmic respiration. In addition, absence of rhythmic respiration would result in the brain becoming hypoxic; generalised hypoxia affects the brain first and if it continues results in death in 4 – 5 min (Ganong 1993).

On average the duration of convulsions were 2.7 min in the present study (experiment 1). Similarly, Casey-Trott *et al* (2013) and Casey-Trott *et al* (2014) found that the average duration of convulsion was 3.8 and 3.4 min, respectively, in neonatal pigs euthanised using a NPCB. The appearance of convulsions can be aesthetically unpleasant and unsettling, however onset of convulsions has been associated with the onset of an isoelectric EEG (Blackmore *et al* 1982, 1984). Gibson *et al* (2009) indicated that isoelectric (or category 2 according to their developed scale) EEG readings were incompatible with awareness. In addition, convulsions occur when modulation of the descending somatomotor activity from the brain by the somatomotor cortex is absent; absence of this activity is a sign of cortical impairment and an indicator of early brain failure (Gregory 2005). Therefore, presence of convulsions, while unsettling to the operator, could potentially be a useful indicator of early brain function failure. In addition, Sanderock *et al* (2014) found loss of muscle tone (e.g. jaw or neck muscles) a reliable reflex measure to distinguish between conscious and unconscious states in poultry. Therefore, presence of convulsions and/or loss of muscle tone could potentially be used as indicators of early brain failure.

The EEG has been used as an indicator of loss of awareness following the application of numerous techniques for euthanasia and pre-slaughter stunning (Blackmore & Newhook 1982; Rault *et al* 2014, 2015). Analysis of the EEG provides information about when undoubted insensibility is present (Blackmore & Newhook 1982) and can contribute to decisions about the onset and duration of insensibility when a particular stunning method is employed (Rault *et al* 2014). In the present study, EEG confirmed that the NPCB resulted in the immediate onset of EEG activity that was not (or not likely to be) compatible with awareness and that EEG activity compatible with awareness did not return in any animal. These EEG results confirm the assumptions afforded by the other variables (e.g. brainstem reflexes, cardiac activity) that the NPCB effectively results in immediate insensibility.

There was no effect of operator on the time to cessation of cardiac activity or duration of convulsions in the present study. In neonatal pigs euthanised using an NPCB, stock person was shown to have an effect on duration of cardiac activity but not convulsions (Casey-Trott *et al* 2013), however all pigs were still made immediately insensible and remained in that state until death. These results suggest that as long as operators are trained properly in the use of the NPCB, this method of euthanasia should be effective at causing immediate and sustained insensibility in goat kids.

To assess the effect of anatomical differences between male and female goats on the ability of the NPCB to cause immediate insensibly and death, both sexes were included in the present study. There was no effect of sex on the time to cessation of cardiac activity or duration of convulsions. These results suggest that in goats up to 48 h of age, this method of euthanasia is effective at causing immediate and sustained insensibility in both sexes.

Conclusion and animal welfare implications

This study demonstrated that an NPCB can successfully be used to euthanise goat kids up to 48 h of age providing the specific anatomical placement and head position are utilised. Goat kids must have their heads bent so that their chins touch the chest and the NPCB must be placed at the back of the goats head between its ears. Furthermore, this study found no effect of operator on efficacy therefore if operators are trained properly in the use of the NPCB then this method of euthanasia should be effective at causing immediate insensibility followed by death in goat kids less than 48 h of age. Finally, the authors caution that there may be differences in operation of different NPCBs, and encourage the validation of other products for use with neonatal goat kids.

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Figure legends

Figure 1. Anatomical placement of the non-penetrating captive bolt (images created by Chelsea Dela Rue).

Figure 2: Cumulative percentage of goats (n = 100) ceasing cardiac activity and convulsions over time. Time point 0 indicates the time immediately following application of the non-penetrating captive bolt.

Figure 3. Electroencephalogram response of goats (n = 7) in the 5 min following application of the non-penetrating captive bolt.

Table 1. Mean number (range) of skull fractures and macroscopic haemorrhage score (range) when the non-penetrating captive bolt was placed on the frontal bone (FRONT; n = 5), on top of the head (POLL; n = 5) or behind the poll between the ears (BACK; n = 5) with the goats lower jaw flat on a firm surface, or behind the poll between the ears with the goats lower jaw touching its chest (BACK-MOD; n = 10).

	Placement of the non-penetrating captive bolt							
	FRONT		POLL		BACK		BACK-MOD	
Fractures (no.)								
Front	2	(2-3)	2	(0-3)	1	(0-4)	2	(0-3)
Back	0	(0-0)	1	(0-4)	2	(0-3)	2	(0-4)
*Subcutaneous haemorrhages (score)**								
Front	2	(1-3)	2	(0-3)	1	(0-3)	2	(0-3)
Back	1	(0-3)	1	(0-3)	2	(0-3)	3	(2-3)
*Subdural haemorrhages (score)**								
Front	2	(1-3)	2	(2-3)	2	(0-3)	2	(1-2)
Back	1	(0-2)	2	(1-3)	2	(2-3)	2	(1-3)

*Haemorrhages were scored based on the macroscopic scoring system described in Casey-Trott *et al* (2013): 1: no haemorrhages present, 2: haemorrhages 0 to 2 cm in diameter, 3: haemorrhages 2 to 4 cm in diameter and 4: haemorrhages > 4cm in diameter.

Table 2. Effect of operator and goat gender on the time to cessation of cardiac activity (P = 0.608 and P = 0.146, respectively) and convulsions (0.807 and P = 0.819, respectively) in goat kids (n = 100) up to 48 h of age after application of a non-penetrating captive bolt

	Number of goats	Time to cessation of cardiac activity (min)	SEM	Time to last convulsion (min)	SEM
Operator					
1	2	7.9	1.63	2.3	2.01
2	8	7.7	0.87	3.3	0.76
3	5	7.8	1.03	3.1	0.91
4	11	9.2	0.68	2.0	0.63
5	5	8.8	1.06	2.3	0.94
6	35	7.6	0.46	2.8	0.41
7	34	8.2	0.45	3.1	0.40
Average		8.2	0.88	2.7	0.87
Gender					
Female	16	7.7	0.67	2.6	0.63
Male	84	8.7	0.36	2.7	0.38

Figure 1.

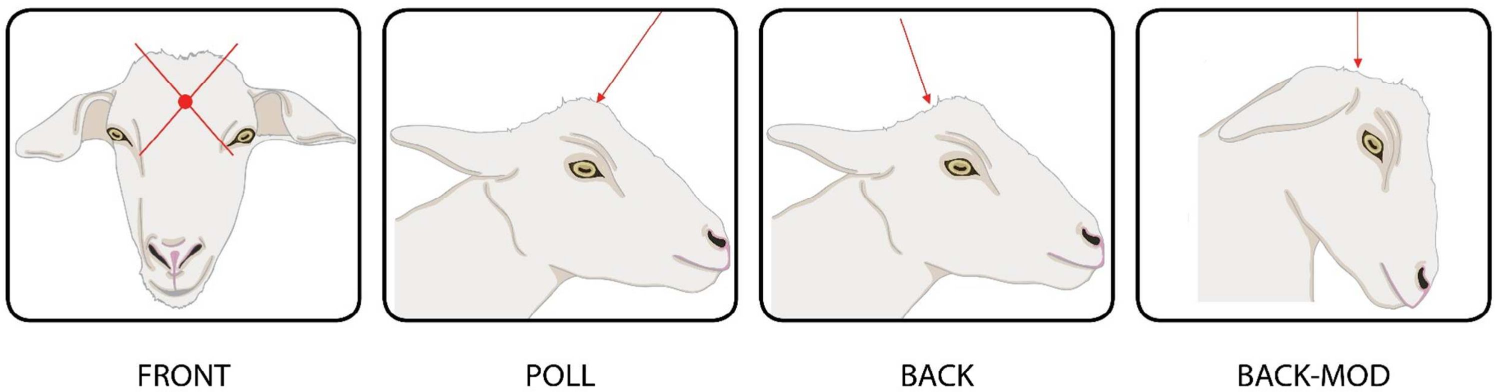


Figure 2.

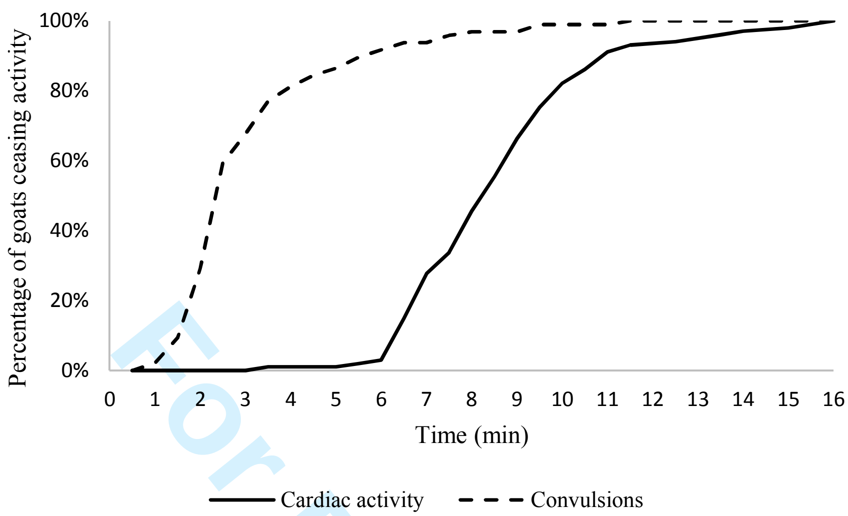
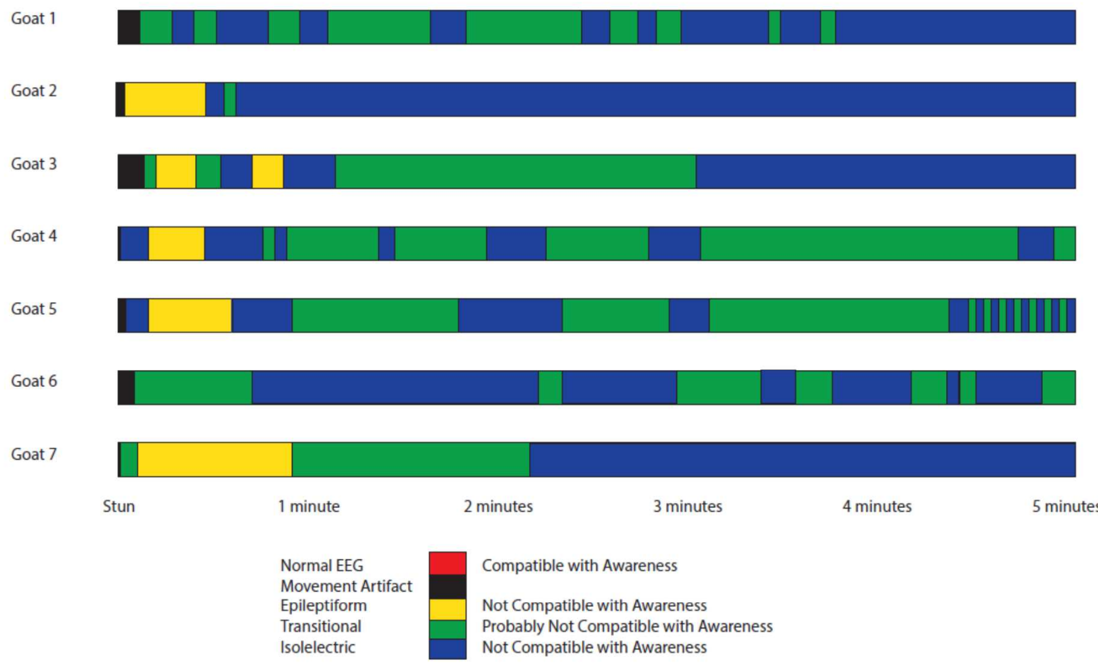

100%
80%
60%
40%
20%
0%
Percentage of goats ceasing activity
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Time (min)
Cardiac activity
Convulsions

Figure 3.


Goat 1
Goat 2
Goat 3
Goat 4
Goat 5
Goat 6
Goat 7
Stun
1 minute
2 minutes
3 minutes
4 minutes
5 minutes
Normal EEG
Movement Artifact
Epileptiform
Transitional
Isolelectric
Compatible with Awareness
Not Compatible with Awareness
Probably Not Compatible with Awareness
Not Compatible with Awareness