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

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3 **Running head:** Euthanasia of goat kids  
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12 **Evaluation of the efficacy of a non-penetrating captive bolt to euthanise neonatal**  
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14 **goats up to 48 hours of age**  
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1  
2  
3 26 **Abstract**  
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5 27 Manual blunt force trauma is a common method of euthanasia or culling of goat kids,  
6  
7 28 however it is difficult to apply consistently and may vary in effectiveness. Therefore, a  
8  
9 29 controlled mechanical method is needed. The overall objective of this research was to  
10  
11 30 evaluate the effectiveness of a non-penetrating captive bolt (NPCB) to euthanize goats up to  
12  
13 31 48 h of age. In a pilot study (n = 27), the optimum anatomical site for placement of the NPCB  
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15 32 was evaluated using signs of insensibility and death, and post-mortem assessment of  
16  
17 33 traumatic brain injury. Three different anatomical sites (frontal bone, poll or behind the poll)  
18  
19 34 were evaluated. In experiment 1 (n=100), goats were euthanized using the optimum  
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21 35 anatomical placement determined in the pilot study and the presence of brainstem reflexes,  
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23 36 rhythmic respiration, convulsions and cardiac activity were recorded. In experiment 2 (n = 7),  
24  
25 37 electroencephalogram (EEG) was recorded to assess awareness following application of the  
26  
27 38 NPCB. Results from the pilot study showed that immediate insensibility followed by death  
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29 39 was achieved when the muzzle of the NPCB was positioned behind the poll and the goat's  
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31 40 head was bent so that the chin touched the chest. In experiment 1, all goats were rendered  
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33 41 immediately insensible without return to sensibility prior to cessation of cardiac activity. In  
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35 42 experiment 2, application of the NPCB resulted in the immediate onset of EEG activity which  
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37 43 was incompatible with awareness. In conclusion, the NPCB reliably caused immediate,  
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39 44 sustained insensibility followed by death in goats up to 48 h of age.  
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46 **Keywords:** animal welfare, electroencephalogram, euthanasia, goats, insensibility, non-  
47 penetrating captive bolt  
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## 49 Introduction

50 Manually applied blunt force trauma (BFT) is a common method of euthanasia and culling  
51 for many neonatal species, including goat kids. However, manually applied BFT is difficult  
52 to apply consistently, is often aesthetically unpleasant for operators to perform and poses a  
53 significant public perception concern. In contrast, mechanically applied BFT performed using  
54 a penetrating (PCB) or non-penetrating captive bolt (NPCB) can deliver an appropriate and  
55 uniform amount of force resulting in more consistent structural damage to the brain (AVMA  
56 2013). Therefore, industry and farm operators have recognised that there is a need to evaluate  
57 mechanical methods of BFT that cause immediate insensibility and death with minimal pain  
58 and distress to the animal.

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

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

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2  
3 74 Health Code (OIE 2015) the optimum placement for hornless sheep and goats is the highest  
4  
5 75 point of the head, on the midline, with the captive bolt aimed towards the angle of the jaw.  
6  
7 76 Alternatively, the Humane Slaughter Association (HSA 2008) recommends that the PCB be  
8  
9 77 placed behind the midline and aimed towards the base of the tongue. However, little is known  
10  
11 78 about the optimal anatomical placement of a NPCB when euthanising neonatal goats.  
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14 79 The objectives of this study were (1) to develop a protocol for NPCB euthanasia of  
15  
16 80 neonatal goats, including the optimum anatomical site for placement of the NPCB muzzle,  
17  
18 81 and (2) evaluate the effectiveness of the NPCB euthanasia protocol in regards to immediate  
19  
20 82 and sustained insensibility and death when applied to goats up to 48 h of age.  
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#### 25 84 **Materials and methods**

26  
27 85 All procedures involving animals were approved by the AgResearch Ruakura Animal Ethics  
28  
29 86 Committees under the New Zealand Animal Welfare Act 1999. The study was conducted  
30  
31 87 between July and August (southern hemisphere winter) 2014 on commercial farms and  
32  
33 88 performed on animals that farmers identified as needing to be euthanised. A pilot study,  
34  
35 89 conducted in two parts, was first performed to aid in the design of the proceeding  
36  
37 90 experimental work.  
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#### 43 92 ***Euthanasia Device***

44  
45 93 A cordless, propane powdered NPCB (TED, BOCK Industries, Inc., Philipsburg, PA, USA)  
46  
47 94 was used to euthanise all goats in the study. The mass of the NPCB bolt was 61.4 g and was  
48  
49 95 released at a velocity of 30.1 m/s. The resulting energy produced by the NPCB bolt was 27.8  
50  
51 96 Joules (R. Bock, personal communication).  
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#### 56 98 ***Pilot study***

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3 99 The aim of the pilot study was to evaluate the optimum anatomical site for placement of the  
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5 100 NPCB muzzle; first by assessing the effect of placement of the NPCB on traumatic brain  
6  
7 101 injury in anaesthetised goats and second, by assessing the effect of placement of the NPCB  
8  
9 102 on signs of insensibility and death.

10  
11 In part 1, fifteen (female, n = 3; male, n = 12) Saanan goat kids (4.0 kg  $\pm$  0.47 SD),  
12  
13 less than 48 h of age, were allocated to one of three treatments (n = 5 / treatment; Figure 1):  
14  
15 1) the muzzle of the NPCB was placed on the frontal bone, at the intersection of two lines  
16  
17 drawn from the lateral canthus of the eye to the region of the horn bud on the opposite side of  
18  
19 the head, and with the lower jaw resting flat on a firm surface (FRONT), 2) the muzzle of the  
20  
21 NPCB was placed on the top of the head (poll) with the lower jaw resting flat on a firm  
22  
23 surface (POLL) or 3) the muzzle of the NPCB was placed behind the poll between the ears  
24  
25 with the lower jaw resting flat on a firm surface (BACK). These anatomical landmarks were  
26  
27 based on published recommendations for placement of a PCB for small ruminants according  
28  
29 to the AVMA (2013), OIE Terrestrial Animal Health Code (OIE 2015) and HSA (2008) for  
30  
31 the FRONT, POLL and BACK locations, respectively.  
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36 114 Goats were weighed, anaesthetised by administering 0.1 mL 2% Xylaxine (Phoenix  
37  
38 Pharm Distributors Limited, Auckland, NZ) intramuscularly. Once anesthesia was confirmed,  
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40 goats were individually placed in a purpose built portable rigid plastic restraint device, which  
41  
42 had four holes in which to place the legs through and a firm surface to support the head. The  
43  
44 restraint device was elevated so the legs of the animals hung down without touching the  
45  
46 ground. The muzzle of the NPCB was positioned according to the allocated treatment and a  
47  
48 single shot was fired. Five min after application of the NPCB, any goats that still had a  
49  
50 heartbeat received an intravenous overdose of pentobarbitone (5 mL; Provet NZ Pty Ltd,  
51  
52 Auckland, NZ), to ensure death.  
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3 123 Following death, goats were dissected by a trained veterinary pathologist blind to the  
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5 124 treatments who scored the number of skull fractures and extent of brain haemorrhaging  
6  
7 125 caused by application of the NPCB. The skin was removed from the dorsal surface of the  
8  
9 126 skull and the amount of haemorrhaging in the subcutaneous tissue was assessed. If fracture  
10  
11 127 lines were visible in the skull they were counted. The head was then disarticulated from the  
12  
13 128 spinal column at the occipitoatlantal joint and cut longitudinally in the midline on a band saw.  
14  
15 129 The extent of intracranial haemorrhage was assessed on the dorsal surface of the brain. The  
16  
17 130 brain was then dissected out and the floor of the cranial cavity was examined for fractures.  
18  
19 131 Haemorrhages underneath the scalp on the dorsal surface of the skull, in the area over the  
20  
21 132 occipital bone, were classified as subcutaneous (SC) haemorrhages. Haemorrhages on the  
22  
23 133 dorsal surface of the brain, after the skull and dura were removed, were classified as subdural  
24  
25 134 dorsal (SDD) haemorrhages. Both SC and SDD haemorrhages were scored based on the  
26  
27 135 macroscopic scoring system described in Casey-Trott *et al* (2013): 1: no haemorrhages  
28  
29 136 present, 2: haemorrhages 0 to 2 cm in diameter, 3: haemorrhages 2 to 4 cm in diameter and 4:  
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31 137 haemorrhages > 4cm in diameter.  
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36 138 In part 2, 12 (female, n = 4; male, n = 8) Saanan goat kids (4.2 kg  $\pm$  0.66 SD), less  
37  
38 139 than 48 h of age, were allocated to one of four treatments (n = 3 / treatment; Figure 1):  
39  
40 140 FRONT, POLL, BACK and BACK-MOD. The NPCB was applied in the same way for  
41  
42 141 FRONT, POLL, and BACK as described in part 1 of the pilot study. The BACK-MOD  
43  
44 142 treatment involved placing the NPCB behind the poll between the ears with the goats head  
45  
46 143 bent so its chin was touching its chest. The purpose of this treatment was to focus the force of  
47  
48 144 the NPCB closer to the thalamus, midbrain and pons regions of the brain.  
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51 145 Goats were weighed then placed individually in the restraint device. The muzzle of  
52  
53 146 the NPCB was positioned according to the allocated treatment and a single shot was fired.  
54  
55 147 Treatments were performed in a random order. Immediately after application of the NPCB  
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3 148 and every 30 seconds thereafter, signs of sensibility (presence of brainstem reflexes) were  
4  
5 149 assessed together with presence of cardiac activity, convulsions and rhythmic respiration,  
6  
7 150 until cardiac activity ceased or for a maximum period of 15 minutes. After application of the  
8  
9 151 NPCB, if any animal showed signs of sensibility (presence of brainstem reflexes or rhythmic  
10  
11 152 respiration) at any stage the NPCB was applied a second time to ensure insensibility followed  
12  
13 153 by death. Brainstem reflexes measured included corneal reflex and response to a painful  
14  
15 154 stimulus. The corneal reflex involved touching the surface of the eye to provoke an eye blink  
16  
17 155 response. To assess the response to a painful stimulus, a needle prick was applied to the nose  
18  
19 156 to provoke a withdrawal response. The presence of rhythmic respiration and convulsions  
20  
21 157 were monitored visually and the presence of cardiac activity was determined by palpation.  
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23 158 Convulsions were defined as the total of clonic and tonic neuromuscular leg spasms (e.g. leg  
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25 159 paddling and rigid leg extensions).  
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### 32 *Experiment 1*

33  
34 162 One hundred (female, n = 16; male, n = 84) Saanan goat kids (3.9 kg  $\pm$  0.60 SD), less than 48  
35  
36 163 h of age, were used to evaluate the efficacy of the NPCB. Goat kids were sourced from four  
37  
38 164 commercial farms. Goat kids were euthanised on-farm by five stock people, who were  
39  
40 165 routinely responsible for euthanising animals' on-farm and who were trained in the use of the  
41  
42 166 NPCB and two science technicians who were trained to use the NPCB. Farm 1 had two stock  
43  
44 167 people and farms 2 and 4 had one stock person per farm involved in the study. All  
45  
46 168 experimental animals were euthanised by the trained technicians on farm 3 and the  
47  
48 169 technicians euthanised 50 – 67% of the goats from farms 1, 2 and 4.  
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51  
52 170 Goats were weighed then placed individually in the restraint device. The BACK-  
53  
54 171 MOD placement for the NPCB was used. This anatomical placement was chosen as it caused  
55  
56 172 immediate insensibility and death in goats in the pilot study. A single shot was fired.  
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3 173 Assessment of insensibility and death was the same as described in part 2 of the pilot study.  
4  
5 174 After application of the NPCB, if any animal showed signs of sensibly at any stage the NPCB  
6  
7 175 was applied a second time to ensure insensibility followed by death. If cardiac activity was  
8  
9 176 still present after 15 min then an overdose of Xylazine (2% Xylazine; Phoenix Pharm  
10  
11 177 Distributors Limited, Auckland, NZ) was administered intramuscularly to ensure death. The  
12  
13 178 first 10 goats euthanised in this study were collected and assessed for traumatic head injury  
14  
15 179 using the same post-mortem methodology described in part 1 of the pilot study.  
16  
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## 21 *Experiment 2*

22  
23 182 Seven male Saanan goat kids, less than 48 h of age, were used to evaluate the effect of the  
24  
25 183 NPCB on latency to loss of awareness.  
26  
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## 30 *Anaesthesia*

31  
32 186 An established minimal anaesthesia model was adapted for use in the goat. Anaesthesia was  
33  
34 187 induced in goat kids using 4% halothane (Halothane-Vet; Merial NZ Limited, Manukau City,  
35  
36 188 NZ) vaporised in oxygen (4 L/min). Once an adequate depth of anaesthesia had been  
37  
38 189 achieved, confirmed by visual inspection, halothane delivery was adjusted to maintain an  
39  
40 190 end-tidal tension of 0.95–1.05 % for the remainder of anaesthesia period. End tidal halothane  
41  
42 191 and CO<sub>2</sub> tension were monitored throughout using an anaesthetic agent monitor (Hewlett  
43  
44 192 Packard M1025B, Hewlett Packard, Hamburg, Germany). Rectal temperature was monitored  
45  
46 193 using a digital thermometer (Q 1437; Dick Smith Electronics, NZ) and body temperature  
47  
48 194 maintained with the aid of a heating pad (T pump; Gaymar Industries Inc., Orchard Park, NY,  
49  
50 195 USA).  
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## 56 197 *EEG recording*

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3 198 Twenty-seven gauge stainless steel needle electrodes (Viasys Healthcare, Surrey, England)  
4  
5 199 were positioned subcutaneously to record EEG and electrocardiograph (ECG) activity. A  
6  
7 200 five-electrode montage was used to record EEG from both the left and right cerebral  
8  
9 201 hemispheres, with non-inverting electrodes placed parallel to the midline over the left and  
10  
11 202 right frontal bone zygomatic processes, inverting electrodes over the left and right mastoid  
12  
13 203 processes and a ground electrode placed caudal to the occipital process (Murrell & Johnson  
14  
15 204 2006). Electrocardiograph was recorded using a base-apex configuration.

16  
17  
18 205 Both EEG and ECG signals were amplified with a gain of 1000 and a band-pass of  
19  
20 206 1.0–500Hz (Iso-Dam isolated biological amplifier, World Precision Instruments Inc.,  
21  
22 207 Sarasota, FL, USA) and digitised at a rate of 1 kHz (Powerlab 4/20, ADInstruments Ltd,  
23  
24 208 Colorado Springs, CO, USA). Once end tidal halothane tension was stable at 0.95–1.05 %,  
25  
26 209 EEG was recorded for 5 min prior to application of the NPCB (baseline) and for a further 10  
27  
28 210 min after application. The NPCB was positioned according to the description in experiment  
29  
30 211 1, and a single shot was fired. The digitised signals were recorded on an Apple Macintosh  
31  
32 212 personal computer for off-line analysis at the conclusion of the experiment.

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35  
36 213 The EEG recorded following the application of the NPCB was compared to the  
37  
38 214 baseline values and in a similar manner to Gibson *et al* (2009). Four categories were  
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40 215 identified: 1) Normal: amplitude and frequency similar to baseline period; 2) Epileptiform:  
41  
42 216 amplitude increased with increased low frequency activity, 3) Transitional: amplitude less  
43  
44 217 than 50% of baseline and 4) Isoelectric: Amplitude less than 12.5% of baseline.

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#### 48 49 219 ***Statistical Analysis***

50  
51  
52 220 Data from the pilot study and experiment 2 are descriptive only and are presented as means  
53  
54 221 and ranges in the table and figures. Data from experiment 1 were tested for homogeneity of  
55  
56 222 variance and normal distribution then subjected to analysis of variance using the mixed  
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3 223 model procedure of SAS version 9.3 (SAS Inst., Inc., Cary, NC, USA). The mixed model was  
4  
5 224 used to test for overall mean differences among operators or goat gender on time to cessation  
6  
7 225 of cardiac activity and convulsions. Farm was included as a random effect. Statistical  
8  
9 226 significance was determined at  $P \leq 0.05$  and  $0.05 < P \leq 0.10$  were considered a tendency.  
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12 227

## 13 14 228 **Results**

### 15 16 229 *Pilot study*

17  
18 230 In part 1, the number of skull fractures and the haemorrhage scores are presented in Table 1.  
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20 231 More skull fractures and higher SC haemorrhage scores were observed at the front of the  
21  
22 232 skull and in the region of the frontal and parietal lobes of the brain when the NPCB was  
23  
24 233 placed on the FRONT or POLL. Conversely, more skull fractures and a higher SC  
25  
26 234 haemorrhage scores were observed at the back of the skull and in the region of the occipital  
27  
28 235 lobe of the brain when the NPCB was placed on the BACK. However, the degree of SDD  
29  
30 236 haemorrhaging was similar in the region of the frontal and parietal lobes of the brain among  
31  
32 237 all three anatomical placements of the NPCB. From these preliminary results it was not  
33  
34 238 evident which anatomical placement would most reliably cause immediate and sustained  
35  
36 239 insensibility and death in goats, therefore the effect of anatomical placement of the NPCB on  
37  
38 240 signs of insensibility and death was assessed in part 2 in conscious goats.

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40  
41 241 In part 2, immediate insensibility was not achieved in any of the three FRONT goats;  
42  
43 242 all three goats exhibited rhythmic respiration and brainstem stem reflexes were also present  
44  
45 243 in one goat after application of the NPCB. After applying the NPCB to the POLL, the first  
46  
47 244 animal became immediately insensible and remained insensible until cessation of cardiac  
48  
49 245 activity, but rhythmic respiration continued in the following two goats after application of the  
50  
51 246 NPCB. Application of the NPCB to the BACK resulted in the first goat becoming  
52  
53 247 immediately insensibility, however the corneal reflex and rhythmic respiration returned 2.5  
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3 248 min after triggering the device. The other two BACK goats became immediately insensible  
4  
5 249 and remained that way until death. Due to the variable results achieved placing the NPCB at  
6  
7 250 the FRONT, POLL or BACK it was decided to position the head so that the goats chin was  
8  
9  
10 251 touching its chest, to more reliably focus the force of the NPCB closer to the thalamus,  
11  
12 252 midbrain and pons regions of the brain. All three BACK-MOD goats became immediately  
13  
14 253 insensible and remained insensible until cessation of cardiac activity.  
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17 254

### 18 255 ***Experiment 1***

19  
20 256 All goats were rendered immediately insensible and no animals showed signs of returning to  
21  
22 257 sensibility prior to death. Only one out of 100 goats needed to be euthanised using an  
23  
24 258 alternative method as cardiac activity had not ceased within 15 minutes; this animal showed  
25  
26 259 no signs of returning to sensibility within that time. The average time to cessation of cardiac  
27  
28 260 activity and convulsions are presented in Table 2. The cumulative percentage of goats ceasing  
29  
30 261 cardiac activity and convulsion was categorized into 1-min intervals and plotted across time  
31  
32 262 (Figure 2).  
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35

36 263 More skull fractures and a higher SC haemorrhage score was observed at the back of  
37  
38 264 the skull and in the region of the occipital lobe of the brain when the NPCB was placed on  
39  
40 265 the BACK-MOD compared with FRONT and POLL (Table 1). More SDD haemorrhaging  
41  
42 266 occurred around the caudal end of the brain, closer to the vital centres of the brainstem.  
43  
44

45 267 There was no effect of operator on the time to cessation of cardiac activity ( $P = 0.608$ )  
46  
47 268 or convulsions ( $P = 0.807$ ; Table 2). In addition, there was no effect of goat gender on the  
48  
49 269 time to cessation of cardiac activity ( $P = 0.146$ ) or convulsions ( $P = 0.819$ ; Table 2).  
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51

52 270

### 53 271 ***Experiment 2***

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3 272 Apnoea, as judged by the absence of carbon dioxide in the respiratory gas sample, developed  
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5 273 immediately following application of the NPCB and persisted for the duration of the  
6  
7 274 recording period in all animals. Changes seen in the EEG following application of the NPCB  
8  
9  
10 275 are illustrated in Figure 3. After a period of movement artefact lasting between 0.7 and 4.2 s  
11  
12 276 (mean 2.1 s), EEG was found to be either epileptiform or isoelectric (definitely not  
13  
14 277 compatible with awareness) in three animals and transitional (probably not compatible with  
15  
16 278 awareness) in the other four animals. The first instance of either epileptiform or isoelectric  
17  
18 279 EEG was seen in all animals between 0.7 and 27.1 s (mean 7.5 s) after application of the  
19  
20  
21 280 NPCB. No periods of normal EEG activity were seen in any animals at any time following  
22  
23 281 application of the NPCB. Changes seen in the EEG for the first 5 min following application  
24  
25 282 of the NPCB are illustrated in Figure 3.  
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## 30 284 **Discussion**

31  
32 285 The word euthanasia is derived from the Greek terms 'eu' and 'thanatos' which combined  
33  
34 286 mean good death. Therefore, the term euthanasia refers to ending an individual animal's life  
35  
36 287 with minimal pain or distress (AVMA 2013). The NPCB caused immediate and sustained  
37  
38 288 insensibility until death, hence a humane death in goat kids up to 48 h of age. However,  
39  
40 289 correct anatomical placement of the NPCB and positioning of the head were important  
41  
42  
43 290 characteristics related to the efficacy of this device. Recommendations regarding the optimal  
44  
45 291 anatomical placement when using a PCB to stun or kill neonatal ruminants (calves, lamb and  
46  
47 292 kids) are available (HSA 2008; AVMA 2013; OIE 2015), however these recommendations  
48  
49 293 are based on research using different PCB/NPCB devices and few studies have investigated  
50  
51 294 the optimum anatomical placement of a NPCB (in particular the TED used in this study) to  
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53  
54 295 euthanise goats up to 48 h of age.  
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3 296 All 100 goats (experiment 1) in the present study were rendered immediately  
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5 297 insensible and remained insensible until death, confirmed using cessation of cardiac activity,  
6  
7 298 when the NPCB was applied behind the poll with the animals chin touching its chest. These  
8  
9  
10 299 results are similar to Casey-Trott *et al* (2013) who found that a NPCB caused immediate  
11  
12 300 insensibility in 100 neonatal pigs less than 3 d of age. These results suggest that applying the  
13  
14 301 NPCB to the back of the kids head (between the ears) with the head bent over is an effective  
15  
16 302 method of euthanasia for goat kids up to 48 h of age.

17  
18 303 In the present study, the extent of traumatic brain injury caused by application of the  
19  
20 304 NPCB to different anatomical sites using sedated animals was initially investigated. Applying  
21  
22 305 the NPCB to the FRONT or POLL of the head caused more haemorrhaging around the  
23  
24 306 frontal region of the brain compared to placing the NPCB behind the midline which caused  
25  
26 307 more damage to the occipital region of the brain, near the thalamus and brainstem. Casey-  
27  
28 308 Trott *et al* (2013) also found that a NPBC caused severe brain haemorrhaging in pigs less  
29  
30 309 than 3 d of age, resulting in significant damage to the brainstem, cortex and subcortical  
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32 310 tissues. These parts of the brain are responsible for vital life functions such as breathing and  
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34 311 therefore should be the areas targeted when using a NPCB. Evaluation of traumatic brain  
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36 312 injury alone was not sufficient to confirm the optimum anatomical placement of the NPCB to  
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38 313 cause immediate and sustained insensibility in goats, therefore the NPCB was applied to the  
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40 314 same three anatomical sites but using conscious animals. It was quickly evident that applying  
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42 315 the NPCB to the FRONT, POLL or BACK locations was not effective, however placing the  
43  
44 316 NPCB behind the midline with the goats head bent was. Correspondingly, Gibson *et al*  
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46 317 (2012) was able to successfully euthanise adult sheep with a PCB placed on the midline of  
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48 318 the head (similarly to the FRONT treatment in the current study). However, it is likely that  
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50 319 the devices used by Gibson *et al* (2012) caused more overall structural brain damage than the  
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3 320 device used in the present study since they were penetrating and/or fired using higher calibre  
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5 321 cartridge charges.

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7 322 Gibson *et al* (2012) also demonstrated the importance of correct placement of the  
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9 323 captive bolt to ensure that animals become insensible immediately and remain in that state  
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11 324 until death. In addition, placement of a PCB was found to be an important factor in causing  
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13 325 sufficient disruption to the brainstem in cattle (Gilliam *et al* 2012). The results from the  
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15 326 current study highlight the importance of evaluating different captive bolt devices on the  
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17 327 species and age group on which they are attended to be used on as it is likely that anatomical  
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19 328 placement of devices and appropriate head position will vary based on the type and power of  
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21 329 the device.

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25 330 Cessation of cardiac activity is often used to confirm death. The average duration of  
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27 331 cardiac activity was 8.2 min in goats less than 48 h of age in the present study. Similar values  
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29 332 were reported for neonatal pigs euthanised with an NPCB; cardiac activity ceased in pigs less  
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31 333 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  
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33 334 (Casey-Trott *et al* 2014). In adult polled ewes, however, cessation of cardiac activity upon  
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35 335 application of a PCB appeared to be more variable (0.25 - 13.7 min; Gibson *et al* 2012).  
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37 336 Continuation of cardiac activity after brain death is due to stimulation from the autonomic  
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39 337 nervous system independent of cerebral regulation (Cooper *et al* 1989). Therefore, although  
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41 338 one goat (1/100 goats) was euthanised using a secondary method due to continuation of  
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43 339 cardiac activity up to 15 minutes after application of the NPCB, it is likely that this animal  
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45 340 was brain dead as during this period brainstem reflexes were absent including rhythmic  
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47 341 respiration. In addition, absence of rhythmic respiration would result in the brain becoming  
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49 342 hypoxic; generalised hypoxia affects the brain first and if it continues results in death in 4 – 5  
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51 343 min (Ganong 1993).  
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3 344 On average the duration of convulsions were 2.7 min in the present study (experiment  
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5 345 1). Similarly, Casey-Trott *et al* (2013) and Casey-Trott *et al* (2014) found that the average  
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7 346 duration of convulsion was 3.8 and 3.4 min, respectively, in neonatal pigs euthanised using a  
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10 347 NPCB. The appearance of convulsions can be aesthetically unpleasant and unsettling,  
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12 348 however onset of convulsions has been associated with the onset of an isoelectric EEG  
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14 349 (Blackmore *et al* 1982, 1984). Gibson *et al* (2009) indicated that isoelectric (or category 2  
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16 350 according to their developed scale) EEG readings were incompatible with awareness. In  
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18 351 addition, convulsions occur when modulation of the descending somatomotor activity from  
19  
20 352 the brain by the somatomotor cortex is absent; absence of this activity is a sign of cortical  
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22 353 impairment and an indicator of early brain failure (Gregory 2005). Therefore, presence of  
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24 354 convulsions, while unsettling to the operator, could potentially be a useful indicator of early  
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26 355 brain function failure. In addition, Sanderock *et al* (2014) found loss of muscle tone (e.g. jaw  
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28 356 or neck muscles) a reliable reflex measure to distinguish between conscious and unconscious  
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30 357 states in poultry. Therefore, presence of convulsions and/or loss of muscle tone could  
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32 358 potentially be used as indicators of early brain failure.

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36 359 The EEG has been used as an indicator of loss of awareness following the application  
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38 360 of numerous techniques for euthanasia and pre-slaughter stunning (Blackmore & Newhook  
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40 361 1982; Rault *et al* 2014, 2015). Analysis of the EEG provides information about when  
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42 362 undoubted insensibility is present (Blackmore & Newhook 1982) and can contribute to  
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44 363 decisions about the onset and duration of insensibility when a particular stunning method is  
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46 364 employed (Rault *et al* 2014). In the present study, EEG confirmed that the NPCB resulted in  
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48 365 the immediate onset of EEG activity that was not (or not likely to be) compatible with  
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50 366 awareness and that EEG activity compatible with awareness did not return in any animal.  
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52 367 These EEG results confirm the assumptions afforded by the other variables (e.g. brainstem  
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54 368 reflexes, cardiac activity) that the NPCB effectively results in immediate insensibility.  
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3 369           There was no effect of operator on the time to cessation of cardiac activity or duration  
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5 370   of convulsions in the present study. In neonatal pigs euthanised using an NPCB, stock person  
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7 371   was shown to have an effect on duration of cardiac activity but not convulsions (Casey-Trott  
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9 372   *et al* 2013), however all pigs were still made immediately insensible and remained in that  
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11 373   state until death. These results suggest that as long as operators are trained properly in the use  
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13 374   of the NPCB, this method of euthanasia should be effective at causing immediate and  
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15 375   sustained insensibility in goat kids.

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18 376           To assess the effect of anatomical differences between male and female goats on the  
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20 377   ability of the NPCB to cause immediate insensibility and death, both sexes were included in the  
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22 378   present study. There was no effect of sex on the time to cessation of cardiac activity or  
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24 379   duration of convulsions. These results suggest that in goats up to 48 h of age, this method of  
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26 380   euthanasia is effective at causing immediate and sustained insensibility in both sexes.  
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### 31 32 382   **Conclusion and animal welfare implications**

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34 383   This study demonstrated that an NPCB can successfully be used to euthanise goat kids up to  
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36 384   48 h of age providing the specific anatomical placement and head position are utilised. Goat  
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38 385   kids must have their heads bent so that their chins touch the chest and the NPCB must be  
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40 386   placed at the back of the goats head between its ears. Furthermore, this study found no effect  
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42 387   of operator on efficacy therefore if operators are trained properly in the use of the NPCB then  
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44 388   this method of euthanasia should be effective at causing immediate insensibility followed by  
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46 389   death in goat kids less than 48 h of age. Finally, the authors caution that there may be  
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48 390   differences in operation of different NPCBs, and encourage the validation of other products  
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50 391   for use with neonatal goat kids.  
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3 463 **Figure legends**  
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7 465 **Figure 1.** Anatomical placement of the non-penetrating captive bolt (images created by  
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9 Chelsea Dela Rue).  
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14 468 **Figure 2:** Cumulative percentage of goats (n = 100) ceasing cardiac activity and convulsions  
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16 469 over time. Time point 0 indicates the time immediately following application of the non-  
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18 470 penetrating captive bolt.  
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23 472 **Figure 3.** Electroencephalogram response of goats (n = 7) in the 5 min following application  
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25 473 of the non-penetrating captive bolt.  
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5 475 **Table 1.** Mean number (range) of skull fractures and macroscopic haemorrhage score (range) when the non-penetrating captive bolt was placed  
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7 476 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  
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9 477 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).  
10

	Placement of the non-penetrating captive bolt							
	FRONT		POLL		BACK		BACK-MOD	
<i>Fractures (no.)</i>								
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)
<i>Subcutaneous haemorrhages (score)*</i>								
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)
<i>Subdural haemorrhages (score)*</i>								
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)

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37 479 \*Haemorrhages were scored based on the macroscopic scoring system described in Casey-Trott *et al* (2013): 1: no haemorrhages present, 2:  
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39 480 haemorrhages 0 to 2 cm in diameter, 3: haemorrhages 2 to 4 cm in diameter and 4: haemorrhages > 4cm in diameter.  
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481 **Table 2.** Effect of operator and goat gender on the time to cessation of cardiac activity ( $P =$   
 482 0.608 and  $P = 0.146$ , respectively) and convulsions (0.807 and  $P = 0.819$ , respectively) in  
 483 goat kids ( $n = 100$ ) up to 48 h of age after application of a non-penetrating captive bolt  
 484

	Number of goats	Time to cessation of cardiac activity (min)	SEM	Time to last convulsion (min)	SEM
<i>Operator</i>					
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
<i>Gender</i>					
Female	16	7.7	0.67	2.6	0.63
Male	84	8.7	0.36	2.7	0.38

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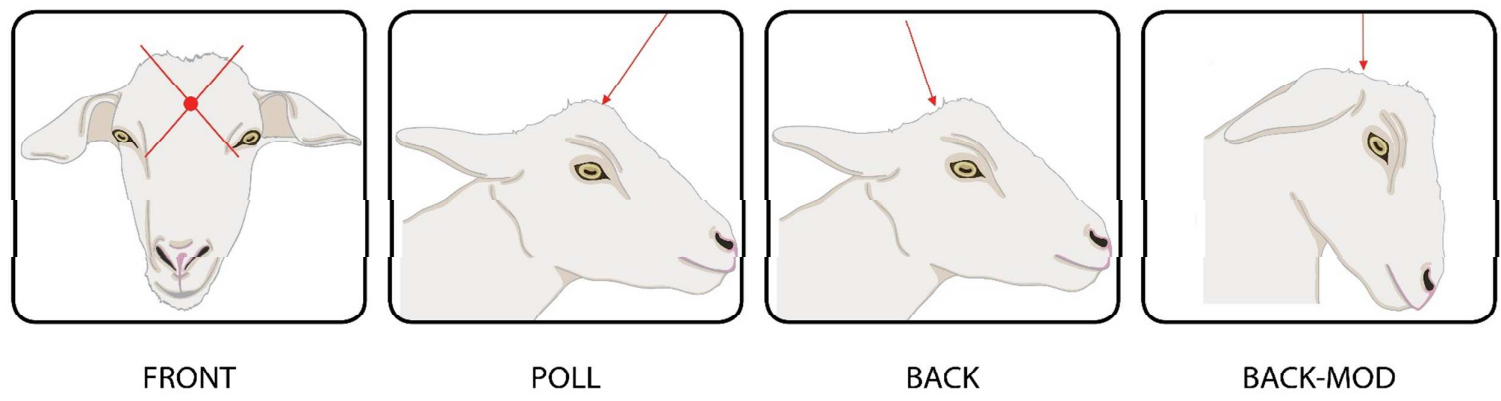
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Figure 1.



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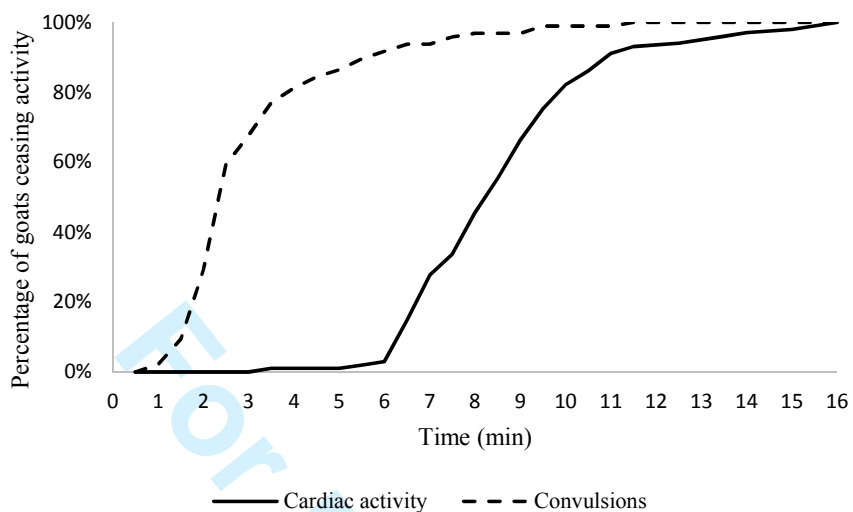
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493 **Figure 2.**



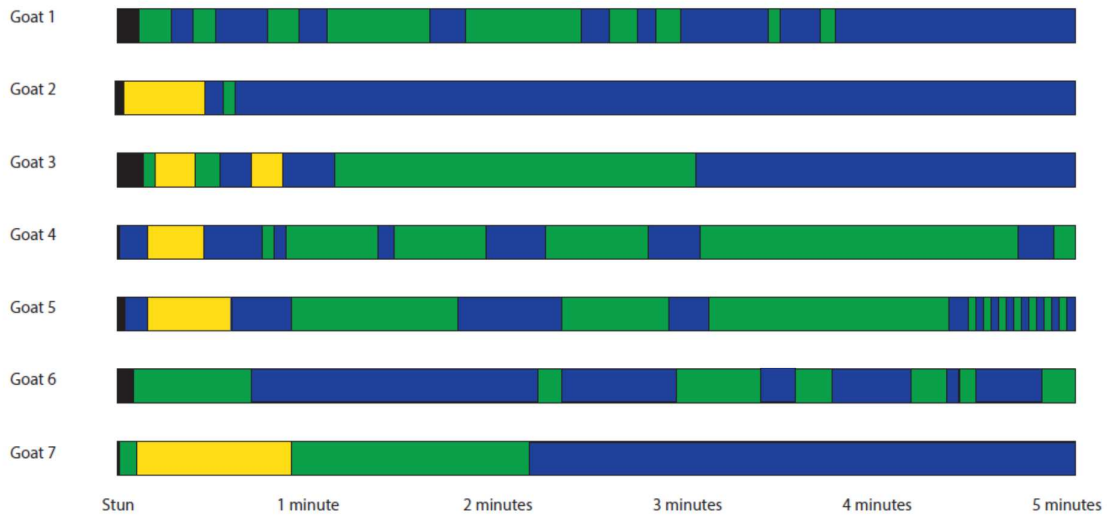
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Figure 3.

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