RESEARCH REPORT
ANIMAL WELFARE

Title: Effectiveness of non-penetrating captive bolt (Zephyr) and restraint for euthanasia of piglets from birth to 9 kg – **NPB # 09-190**

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INDUSTRY SUMMARY

Timely euthanasia of low birth weight and compromised piglets prevents unnecessary suffering, provides littermates with a greater chance at success, and offers some economic benefits by reducing continued care and feed costs. Blunt force trauma (BFT) remains the most commonly practiced method for euthanasia of piglets; but the effectiveness of the technique is significantly limited by the size of the piglet, and is not recommended for piglets greater than 12 lbs. When properly performed BFT has been shown to be humane, although it may not be aesthetically or emotionally acceptable for many stock people. Therefore alternative euthanasia methods for piglets are being investigated. Ideally, an alternative should be consistent, humane, aesthetically acceptable, and capable of being used effectively for piglets from birth through the start of nursery phase. Previous investigation (NPB Project #06-165) of a pneumatic non-penetrating captive bolt gun (NPCB) powered by an air compressor showed promise but the design required some modification to ensure consistency. The project reported here investigated the effectiveness of the modified design for euthanasia of newborn piglets and its potential to be used on larger piglets up to 9 kg. (20 lbs).

The present study consisted of two trials. In Trial 1 we tested the effectiveness of the NPCB on 100 suckling piglets that were less than 3 days of age (average weight 1.04 kg ± 0.03 SE) using reflexes that indicate signs of consciousness, duration of neuromuscular spasms (leg paddling) and time to cardiac arrest. We also determined

the degree of brain damage by macroscopic scoring of damage at gross necropsy, and by CT scans performed by a veterinary radiologist and microscopic examinations of the brain by a veterinary pathologist on a sample of piglets. Ten stock people (10 piglets each) from 4 different farms performed the euthanasia.

All 100 piglets were immediately rendered insensible without return to consciousness. On average, leg movements ceased in 229 sec (± 9.2 SE). Only five piglets (5%) required a secondary step due to prolonged presence of a heartbeat; all other piglets reached full cardiac arrest in 444 sec (± 18.7 SE). From CT scans, skull fracture displacement averaged 6.2 mm (± 0.07 SE) and severe hemorrhage was reported in 50% of the piglets scored. From macroscopic scoring, moderate to severe damage was found in all piglets. Microscopic analysis reported moderate hemorrhage on the brain surface in 70% of the piglets scored as well as moderate hemorrhage within the brain tissue in 50% of the piglets scored. These results indicated that method was highly effective for euthanasia of the neonate piglets and that its effectiveness on larger weight classes should be investigated.

In Trial 2, 5 piglets were selected from each of four weight classes (n=5: 3, 5, 7, and 9 kg). All piglets were anaesthetized prior to application of the NPCB. Piglets were monitored for rhythmic breathing, leg spasms, and heartbeat. Port-mortem CT scan, macroscopic, and microscopic scoring analyses were completed for all 20 piglets to assess the amount of brain damage and to compare it with that found in the neonates. Of the 20 piglets tested, one required an extra shot due to the presence of rhythmic breathing. Breathing was immediately absent in the remaining 19 piglets. Leg spasms ceased on average in 148 sec (± 12.4 SE). A secondary step was required for one piglet due to sustained presence of a heartbeat. All other piglets reached full cardiac arrest in 371 sec (± 17.9 SE). From post-mortem CT scan analysis, fracture displacement averaged 9.38 mm (± 0.84 SE) and mild hemorrhage severity was reported in 65% of the piglets. From macroscopic scoring, moderate to severe damage was reported in > 90% of piglets.

Comparisons between the neonatal and larger anesthetized piglets indicated that duration of leg spasms was significantly shorter in the larger anaesthetized piglets ($p < .001$); but time to full cardiac arrest was not different. Results of CT scans and microscopic scoring indicated that brain hemorrhage was significantly less severe in the larger, anaesthetized piglets but that skull fracture displacement was significantly greater ($p=.019$).

The results form Trial 2 indicated that the NPCB can effectively induce cardiac arrest in piglets up to 9 kg and that it caused brain lesions known to be associated with loss of consciousness in Trial 1. The next stage of research will be testing the effectiveness of the NPCB on conscious piglets. Due to the size of the piglets within

the next trial (up to 9 kg), a restraint device will also be designed to ensure the safety of the operator and the piglet.

KEY WORDS: euthanasia, piglet, non-penetrating captive bolt, brain lesions

SCIENTIFIC ABSTRACT

The overall objective of this project is to test the effectiveness of a non-penetrating captive bolt (Zephyr) for the euthanasia of piglets up to 9 kg (20 lbs). The study was completed in two trials. In Trial 1 we tested the effectiveness of the NPCB on 100 low viability piglets < 72 hrs of age (1.04 kg ± 0.03 SE), Piglets were monitored continuously for rhythmic breathing, neuromuscular leg spasms, and heartbeat until full cardiac arrest (FCA). Brainstem and spinal reflexes were used to assess sensibility from the time of application until the end of leg spasms. Macroscopic scoring was done at necropsy on all 100 piglets. CT scans and microscopic analyses of sectioned and stained brains from a sample of 10 piglets were also used to assess degree of skull fracture and brain hemorrhage. All 100 piglets were immediately rendered insensible without return to consciousness. Leg spasms ceased in 229 sec (± 9.2 SE) and time to FCA was 444 sec (± 18.7 SE). Five piglets required a secondary step (exsanguination) due to a sustained heartbeat for > 10 minutes. Skull fracture displacement (FD) averaged 6.2 mm (± 0.07 SE) and a severe hemorrhage severity (HS) score was observed in 50% of piglets scored from by CT scans. Macroscopic scoring indicated moderate to severe damage in 100% of piglets, and histological analysis found moderate subdural (HSD) and parenchymal (HP) hemorrhage in 70% and 50% of piglets, respectively.

In Trial 2 we applied the NPCB to five piglets in each of four weight classes (3, 5, 7, and 9 kg) Since this was a novel technique for piglets > 5 kg, the piglets were first anaesthetized with 71.4 mg/ml ketamine, 14.3 mg/ml xylazine and 1.4 mg/ml butorphanol 0.2 mL/kg IM to eliminate pain and distress if the technique was not effective. Only one piglet (at 8.7 kg) required an additional shot due to presence of rhythmic breathing. Breathing was immediately absent in all other piglets. Leg spasms ceased in 148 sec (± 12.4 SE). A secondary method (sodium pentobarbital 100 mg/kg) for euthanasia was required for one piglet due to sustained heartbeat. All other piglets reached FCA in 371 sec (± 17.9 SE). FD averaged 9.38 mm (± 0.84 SE) with mild HS in 65% of piglets scored. Moderate to severe macroscopic damage was reported in ≥ 90% of piglets. 50 % of piglets had mild and 50 % had moderate HSD . Only one piglet had no parenchymal HP (5.0 kg) although parenchymal hemorrhage was detected in this same piglet on its CT scan. In comparison to the brain lesions of neonatal piglets, damage was less severe in the anaesthetized piglets (Mann-Whitney test for ordinal data: HS: p=.001, HSD: p=0.007; HP: p=0.041) despite greater FD (t-test: p=.019). Although brain damage in the

anaesthetized piglets was less severe than that of the conscious neonates, the Zephyr still caused parenchymal brain lesions and effectively induced cardiac arrest in all but one piglet. The next trial will test the effectiveness of the NPCB on conscious piglets up to 9 kg.

INTRODUCTION

Within litter bearing species, including swine, it is not uncommon to see variability in size, weight, and vigour among littermates. Euthanasia of the low-viability or moribund neonatal piglets is common practice on commercial swine farms. Currently, the pre-weaning mortality rate in the United States is 12.2% (PigCHAMP, 2009). Timely euthanasia prevents unnecessary suffering of the piglet and provides economic benefit to the producer by reducing continued care and feed costs (Morrow et al., 2006; Fix et al 2010). Low birth weight piglets are often selected for euthanasia due to low pre-weaning and nursery survival rates (Smith et al., 2007). Those that do survive tend to be of poor quality at finishing, thereby reducing overall profit return from the herd (Fix et al., 2010). Removal of those compromised piglets offers the remaining piglets a greater chance at survival, and improves the overall well-being of the herd (Morrow et al., 2006).

When euthanasia is necessary, it is essential that the process be carried out in a manner that minimizes the pain and distress of the animal (AVMA, 2007). The chosen method should be practical, repeatable, safe, and consistently render the animal immediately insensible to ensure no pain is felt until death. Brainstem and spinal reflexes can be used to determine if an animal is conscious (Erasmus et al., 2010a).

Recommended methods for euthanasia of piglets < 12 lbs include blunt force trauma (BFT), carbon dioxide (CO2), non-penetrating captive bolt (NPCB), electrocution (piglets > 10 lbs), and anaesthetic overdose (NPB, 2009). BFT is the most commonly practiced method. Although research has shown the method to be humane (Widowski et al., 2008), it is not aesthetically or emotionally acceptable for many stock people. Furthermore, for piglets between 12 and 20 lbs, BFT is not an option, leaving the producer with few practical alternatives (gunshot, penetrating captive bolt, CO2, electrocution, anaesthetic overdose). These piglets are often part of the transition phase from farrowing to nursery status, a critical period for euthanasia. In an attempt to provide a practical, reliable method for piglets up to 9 kg (20 lbs), the NPCB is being further examined for effectiveness on piglets at this stage.

Previous investigation (NPB Project #06-165) of a pneumatic non-penetrating bolt gun powered by an air compressor (the Zephyr) showed promise but the design required some modification to ensure consistency. The project reported here investigated the effectiveness of the modified design for euthanasia of newborn piglets and

its potential to be used on larger piglets up to 9 kg. (20 lbs). The study was conducted in two trials. The first trial assessed the effectiveness of the Zephyr for piglets < 72 hrs of age, by monitoring signs of sensibility and time to death, followed by post-mortem assessment of brain and skull lesions through CT scans, macroscopic scoring, and histological analysis. The second trial tested the ability of the Zephyr to cause death in anaesthetized piglets up to 9 kg. Since this was a novel test for piglets > 12 lbs, all piglets in the second trial were anaesthetized to ensure no pain was felt by the animal. The estimated times to brain death and cardiac arrest and the degree of brain lesions in the anaesthetized piglets were compared to those of neonates in order to determine the potential for the method to be used on conscious piglets up to 9 kg.

OVERALL OBJECTIVES:

a. Determine the maximum weight of suckling pigs that the Zephyr non-penetrating captive bolt effectively induces rapid loss of consciousness.
b. Determine the maximum weight of suckling pigs that the Zephyr non-penetrating captive bolt effectively achieves death quickly and consistently.
c. Develop restraint techniques that support application of the Zephyr

A number of steps were outlined in order to achieve these overall objectives. The first step was to test the modified design on conscious neonates. The second step was to determine the potential for its use on larger piglets. If results from Step 2 indicated significant brain trauma in larger weight classes of piglets, we would proceed to the next steps: 3. determining the effectiveness on the larger weight classes of conscious piglets, and finally; 4. developing restraint methods for ease of application. This first year of funding was for steps 1 and 2 and those results are reported here. We have secured matching funds for proceeding to Steps 3 and 4.

EQUIPMENT

The non-penetrating captive bolt (Zephyr) used in this study was developed by the Ontario Ministry of Agriculture, Food and Rural Affairs and has been previously tested for the euthanasia of turkeys (Erasmus et al., 2010b; Erasmus et al., 2010c). The Zephyr consisted of a pneumatic nail gun (NS 100A ¼" Narrow Crown Stapler, Port Cable, Jackson, TN) retrofitted with a convex nylon bolt head (diameter: 25mm, length: 38mm) attached to a cylindrical metal bolt (diameter: 8 mm). The nylon head was recessed 33 mm from the end of the gun barrel. When the bolt was fully extended, the nylon head protruded 17 mm past the gun barrel and could

travel a maximum distance of 50 mm. The compressor powering the gun was set to deliver a pressure force of 115 to 120 PSI.

Trial 1: Neonates

MATERIALS AND METHODS

Ten experienced stock people from four different farms, one research station (R1) and three commercial farms (C1, C2, C3), each euthanized 10 low-viability piglets using the Zephyr. Prior to euthanasia, each stock person was trained on how to use the Zephyr. All 100 piglets were < 72h of age (average weight 1.04 kg ± 0.03 SE) and were either non-viable, moribund or injured. The piglets were manually restrained in the sternal position on a hard flat surface. The Zephyr was applied to the frontal bone between the eyes and fired twice in rapid succession, followed by one shot to the back of the skull behind one ear. Immediately following application, piglets were assessed for signs of consciousness using brainstem and spinal reflexes: presence or absence of corneal reflex, pupillary light response, jaw tone, and response to nose prick. Clonic and tonic neuromuscular spasms (convulsions) were monitored along with the presence of breathing and heartbeat. Reflexes were repeatedly checked in that order every 15 seconds until cessation of neuromuscular spasms. Termination of tonic convulsions was used to estimate time of brain death (ETOD). A secondary step of exsanguination was used for piglets with sustained presence of convulsions or heartbeat for > 10 min. Time to full cardiac arrest (FCA) was recorded when a discernable heartbeat was no longer present. A brief questionnaire was provided to each stock person to assess individual swine experience and rate the method on a 10 point scale (1 = completely ineffective → 10 = completely effective).

All 100 piglets were macroscopically scored to assess skull fracture and hemorrhage at necropsy (Table 1). Post-mortem CT scans and histological scoring were completed on a sample of 10 piglets. CT scans were scored by a veterinary radiologist. Hemorrhage severity (HS) was scored subjectively on a scale from 0-3: (0 = none, 1 = mild, 2 = moderate, 3= severe). Fracture displacement (FD) was quantitatively determined by measuring skull fragment displacement (in mm) from the intact position. The brains from the piglets selected for CT scans (n=10) were removed and fixed in 10% buffered formalin for 7 days for histological analysis. Sections were taken from the cerebral cortex, midbrain, and brainstem for each of the 10 brains. Slides were stained with hematoxylin and eosin followed by microscopic scoring completed by a veterinary pathologist. Subdural (HSD) and parenchymal (HP) hemorrhage scores were recorded on a scale from 0-4 (0 = none present, 1 = minimal < 5%, 2 = mild (up to 10%), 3 = moderate (up to 30%), 4 = severe (> 30%)) based on the amount of hemorrhage coverage.

Analysis of variance using SAS Mixed model was used to test whether there was a linear effect of order of piglets euthanized by an individual stock personnel, with age, weight and farm as covariates. The average duration of leg spasms and heart beat for the piglets euthanized by a stock person was compared to overall group average using t-tests.

RESULTS

All 100 piglets were immediately rendered insensible without return to consciousness as determined by the absence of the corneal and pupillary light reflex, as well as the lack of breathing, jaw tone, and response to a nose prick. Five piglets required a secondary step due to sustained presence of a heartbeat. ETOD averaged 229 sec (± 9.2 SE). Time to FCA averaged 444 sec (± 18.7 SE). Trial duration results for average clonic and tonic leg spasms, as well ETOD and FCA are reported in Table 2. On average, stock personnel rated the Zephyr at 8.7 ± 1.6 in terms of effectiveness, with the only score lower than 7 from a worker with < 1 year experience with swine.

With age, weight, and farm as covariates, no overall effect was found for piglet order of euthanasia on duration to full cardiac arrest p=0.737. However, there was a farm by order interaction (p=0.025) with the first farm that was tested. The effect of piglet order on time to full cardiac arrest was approaching significance for R1 (p=0.061).

Figure 1 shows the average duration of time between application of the NPCB and the end of all neuromuscular leg spasms and end of heartbeat for piglets euthanized by individual stock people in comparison to the group average. The average duration of leg spasms of piglets euthanized by individual stock people was never significantly different from the group average. The average duration of heartbeat for each piglet euthanized by individual stock people was only different from the group average in the case of stock person 9. The end of heartbeat for piglets euthanized by stock person 9 (315.6 sec ± 38.0 SE) was significantly shorter than the group average (444.6 sec ± 18.6 SE; p=0.03).

Data from CT scans indicated that FD averaged 6.2 mm (± 0.07 SE) and the most frequent score for HS was 3 (severe) at 50%. The most frequent macroscopic scores were: SK score of 3 at 68%, SC score of 5 at 35%, SD-D score of 5 at 35%, and SD-V score of 5 at 42% (Table 3). From analysis of microscopic scoring, the most frequent scores for hemorrhage were: HSD score of 3 (moderate) at 70% and HP score of 3 (moderate) at 50%.

Trial 2: Anesthetized piglets

MATERIALS AND METHODS

Five piglets were selected from each of four weight classes: (n=5) 3, 5, 7 and 9 kg. One stock person, experienced with the Zephyr from Trial 1, euthanized the 20 piglets. The piglets were anaesthetized with Modified Swine Premix (71.4 mg/ml ketamine, 14.3 mg/ml xylazine and 1.4 mg/ml butorphanol 0.2 mL/kg IM). After the piglets reached a moderate plane of anaesthesia as determined by nose prick and pedal reflex, they were held in sternal recumbancy on a surgical table and the NPCB was applied as described in Trial 1. Piglets were monitored for breathing, heartbeat, and neuromuscular convulsions until full cardiac arrest. End of tonic convulsions was used to estimate time of brain death. Post-mortem CT scans, macroscopic scoring and histological analysis was completed for all 20 piglets to assess skull fracture and brain lesions. All scoring was completed as described in Trial 1.

RESULTS

One 8.7 kg piglet required an extra gunshot following a misfire which resulted in the presence of rhythmic breathing. Breathing was immediately absent in the remaining 19 piglets. Neuromuscular leg spasms ceased on average in 148 sec (± 12.4 SE). A secondary method (sodium pentobarbital 100 mg/kg) was necessary for one 2.5 kg piglet due to sustained presence of a heartbeat. All other piglets reached full cardiac arrest in 371 sec (± 17.9 SE). Trial duration results for average clonic and tonic leg spasms, as well ETOD and FCA are reported in Table 2.

From post-mortem CT scan analysis, FD averaged 9.38 mm (± 0.84 SE) and the most frequent HS score was 1 (mild) at 65%. From macroscopic scoring the most frequent scores were: SK score of 3 at 60%, SC score of 4 at 45%, SD-D score of 5 at 50%, and SD-V score of 4 at 45% (Table 4). From histological analysis, the most frequent scores for hemorrhage were: HSD score of 2 and 3 (mild to moderate) at 50% and HP score of 1 and 2 (minimal to mild) at 45%.

Comparison of Trial 1 and Trial 2

Both the veterinary radiologist and veterinary pathologist were blind to the weight class of piglet from which the samples were obtained. Mann-Whitney tests were used for ordinal data and t-tests were used for FD, ETOD and FCA in order to test for differences between piglets in Trials 1 and 2. ETOD was significantly shorter in the anaesthetized piglets ($p < 0.001$), but differences in time to FCA were not significant. Brain lesions were significantly less severe in the anaesthetized piglets: HS $p=0.001$; SD-V $p<0.001$; HSD $p=0.007$; HP $p=0.041$. There were no significant differences in SC, SK, or SD-D between the piglets in the two trials: $p=0.165$,

p=0.397, p=0.146 but FD was significantly higher in the anaesthetized piglets compared to the neonates: p=0.019.

DISCUSSION

The results from Trial 1 indicate that the modified design of the Zephyr is highly effective for the euthanasia of neonatal piglets < 72 hours of age. The method caused immediate and irreversible insensibility in all 100 piglets and induced a timely death as seen by the duration of leg spasms and heartbeat. The final end time of leg spasms was used to estimate time of brain death as has been done on previous studies on poultry (Dawson et al., 2007; Dawson et al., 2009; Erasmus et al 2010b). In 95% of piglets euthanized, the Zephyr was used effectively without a secondary step. Although the remaining 5% of piglets had a sustained rhythmic heartbeat at the 10 minute cut-off point, the heartbeat was reported as faint and slower than the initial heart rate taken before Zephyr application. Overall, stock personnel rated the Zephyr as highly effective, but most still preferred their current method, BFT. Many expressed interest in testing the Zephyr on larger piglets, where BFT was considered to be more difficult.

The analysis for order effect was used to determine whether there was a 'learning curve' associated with the use of the Zephyr. The heartbeat duration of each of the 10 piglets euthanized by an individual stock person were analyzed in terms of piglet order from first piglet to last for that stock person. Overall, no order effect was found, suggesting each stock person was capable of effectively using the Zephyr consistently from the first piglet to the last. There was an interaction effect approaching significance for piglet order and farm for R1; however, R1 was the first farm on the trial and the differences are likely due to improvements made to the data collection techniques and the training process. In terms of the repeatability of the Zephyr, even though there was variation in duration of piglet leg spasms and heartbeat between operators, only one stock person had statistically significant differences from the group mean; therefore, the Zephyr appears to be easily learned and reliably operated.

CT scan, macroscopic, and histological analyses reported moderate to severe damage in all piglets. Subdural and parenchymal brain lesions were reported. Severe subdural hemorrhage causes brain damage by a build-up of pressure within the brain case whereas parenchymal hemorrhage is responsible for direct damage to brain tissue essential for life. Based on the reflex observations as well as the substantial damage to the skull and brain of neonatal piglets within Trial 1, the Zephyr inflicts sufficient damage to cause irreversible brain damage and induce a timely death.

The results from Trial 2 indicated that the Zephyr can effectively induce cardiac arrest in piglets up to 9 kg. Since all piglets were anaesthetized prior to Zephyr application, it was not possible to monitor the brainstem and

spinal reflexes for signs of sensibility. One 8.7 kg piglet required a second shot due to the return of rhythmic breathing. The second shot was effective and the piglet progressed to a timely death. Based on video of the event, the initial firing of the Zephyr appeared to be a misfire, as the barrel was not flush with the forehead; however, the size of the piglet may have influenced the efficacy of the Zephyr. As in Trial 1, only 5% of piglets required a secondary step, in this case one piglet weighing 2.5 kg. Leg spasms ceased on average in 2 minutes and 30 seconds with the hearts stopping in just over 6 minutes.

Damage to the skull and brain tissue based on CT scan, macroscopic and histological analyses, reported mild to moderate damage. The majority of macroscopic scores (90%) reported moderate to severe damage; whereas, CT scan HS and histological HSD and HP reported predominantly mild to moderate damage. CT scan and histological analyses may be considered more useful since these methods have been shown to be more sensitive (Erasmus et al 2010c) and less likely to be biased as they were completed by trained personnel blind to the weight class of piglet.

Comparing the two trials, there was an overall trend for less severe lesions in the anaesthetized piglets. Some differences were statistically significant, such as HS, SD-V, HSD, and HP; whereas, other differences were not statistically significant (SC, SK, SD-D), but still followed the trend of less severe damage in the anaesthetized piglets. FD was the only result found to be significantly greater in the anaesthetized piglets. This may be due to the difference in skull thickness and calcification between the neonates, average weight 1.04 kg (± 0.03 SE) all < 3 days of age, and the anaesthetized piglets all of whom were > 3 kg and between 7 and 28 days of age. Although estimated time of brain death (ETOD) and time to full cardiac arrest (FCA) were shorter in the larger, piglets, these results may be confounded by the effects of the anaesthesia. While this major difference of the trials should be considered when considering the compared results, it is important to remember that the primary goals of the anaesthesia trial were to determine if cardiac arrest was consistently achieved by the Zephyr and to gain an understanding of the amount of damage the Zephyr inflicted as piglet size increased. Results from both Trial 1 and Trial 2 showed promising results for use of the Zephyr for the euthanasia of conscious piglets up to 9 kg.

The next stage of research will be testing the effectiveness of the Zephyr on conscious piglets up to 9 kg by monitoring signs of sensibility and post-mortem analysis of brain and skull lesions. Due to the significant increase in size of the piglets within the next trial, a restraint device will also be designed to ensure the safety of the operator and the piglet.

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Table 1: Scoring system used for macroscopic scoring of skull fracture (SK) and subcutaneous (SC), subdural-dorsal (SD-D), and subdural-ventral (SD-V) hemorrhage in both Trial 1 and Trial 2 (adapted from Veltri and Klem 2005)

Description of Macroscopic Scoring System	
Score	**Definition**
SK	
1	No fractures, intact skull
2	Hairline fractures, no separation of bone
3	One to two complete, fully separated fractures or a single depressed fracture
4	More than just a single depressed fracture, 3 to 5 complete fractures
5	More than five complete fractures, fully fragmented skull
SC, SD-D, and SD-V	
1	None
2	Some bleeding, less than 25% of surface area
3	Bleeding between 26-50% of surface area
4	Bleeding between 51-75% of surface area
5	Severe bleeding, 76-99% of surface area
6	Completely covered

Table 2: Average durations (sec ± SE) of clonic leg spasms, tonic leg spasms, estimated time of brain death, and full cardiac arrest based on the average weight (± SE) of each group. Trial 1 n=100 and Trial 2 n=5 for each weight class.

Average Durations (sec ± SE) based on Average Weight					
	Trial 1: Neonate	**Trial 2: Anaesthetized**			
	1.04 ± 0.03 kg	**3.0 ± 0.18 kg**	**4.84 ± 0.05 kg**	**7.15 ± 0.09 kg**	**8.76 ± 0.12 kg**
Clonic Duration (s)	101.5 ± 7.4	51.0 ± 6.4	57.4 ± 3.6	64.8 ± 4.1	85.4 ± 17.6
Tonic Duration (s)	128.1 ± 6.5	115.2 ± 49.9	79.6 ± 7.1	77.2 ± 22.8	64.6 ± 8.0
Estimated time of brain death (s)	229.7 ± 9.2	171.5 ± 53.6	137.0 ± 6.2	142.0 ± 24.3	150.0 ± 16.5
End of heartbeat (s)	444.6 ± 18.7	424.5 ± 85.5	402.2 ± 43.3	359.0 ± 18.5	368.6 ± 44.3

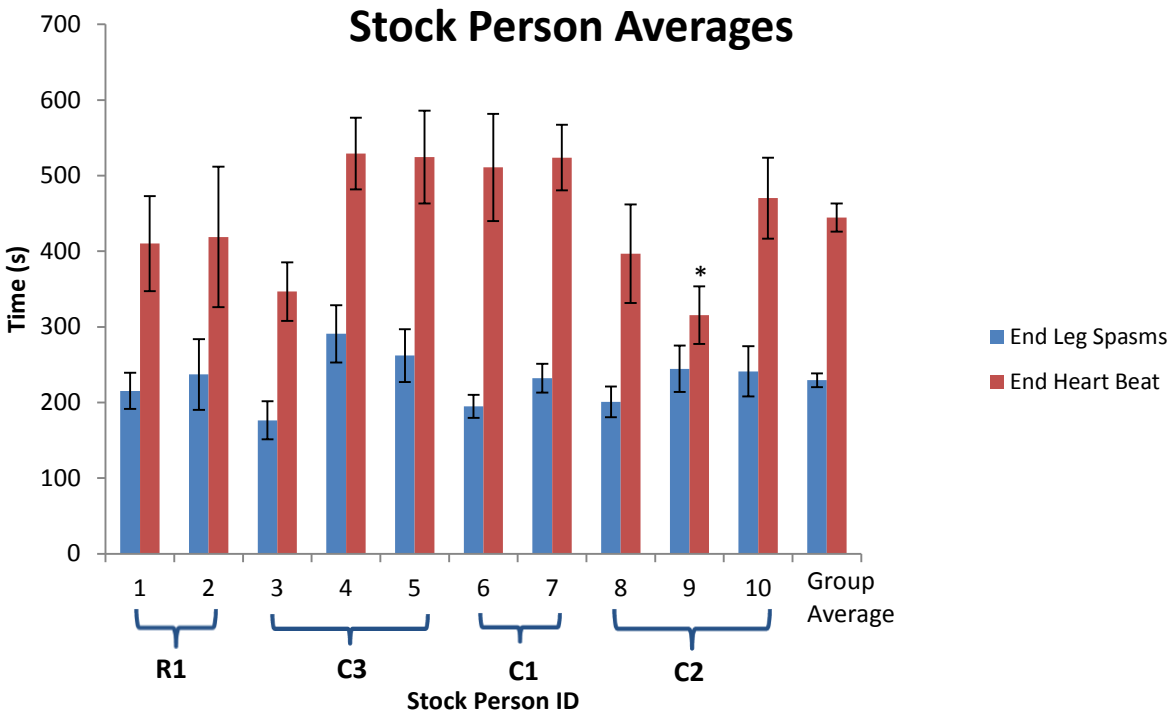

Figure 1. Average duration of leg spasms and heartbeat of piglets euthanized by individual stock people compared to the overall group average. Significant differences from the group average ($p < 0.05$) noted by (*). Average ± SE.

Table 3: Frequency distribution of macroscopic scores from Trial 1. Scores based on scoring system described in Table 2. Highlighted cells represent score with highest frequency for each category.

Trial 1 Frequency Distribution of Macroscopic Scores						
	Score of 1	**Score of 2**	**Score of 3**	**Score of 4**	**Score of 5**	**Score of 6**
Skull Fracture (SK) n=100	0%	0%	67.0%	33.0%	0%	N/A
Subcutaneous (SC) n=100	0%	3.0%	22.0%	21.0%	35.0%	19.0%
Subdural-Dorsal (SD-D) n=99	0%	1.0%	22.2%	34.3%	35.4%	7.1%
Subdural-Ventral (SD-V) n=94	0%	0%	18.1%	33.0%	40.4%	8.5%

Table 4: Frequency distribution of macroscopic scores from Trial 2. Scores based on scoring system described in Table 2. Highlighted cells represent score with highest frequency for each category.

Trial 2 Frequency Distribution of Macroscopic Scores						
	Score of 1	**Score of 2**	**Score of 3**	**Score of 4**	**Score of 5**	**Score of 6**
Skull Fracture (SK) n=20	0%	5.0%	60.0%	30.0%	5.0%	N/A
Subcutaneous (SC) n=20	0%	5.0%	15.0%	45.0%	30.0%	5.0%
Subdural-Dorsal (SD-D) n=20	0%	1.0%	10.0%	30.0%	50.0%	10.0%
Subdural-Ventral (SD-V) n=20	0%	10.0%	30.0%	50.0%	10.0%	8.5%