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# Effectiveness of a nonpenetrating captive bolt for euthanasia of 3 kg to 9 kg pigs[1]

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**ABSTRACT:** The objective of this study was to determine the effectiveness of a nonpenetrating captive bolt, Zephyr-E, for euthanasia of suckling and weaned pigs from 3 to 9 kg (5–49 d of age) using signs of insensibility and death as well as postmortem assessment of traumatic brain injury (TBI). The Zephyr-E was used by 15 stock people to euthanize 150 compromised pigs from 4 farrowing and nursery units from commercial farms and 2 research stations. Brainstem reflexes, convulsions, and heartbeat were used to assess insensibility, time of brain death, and cardiac arrest following Zephyr-E application. Skull fracture displacement (FD) was quantified from computed tomography (CT) scans ($n = 24$), macroscopic scoring was used to assess brain hemorrhage and skull fracture severity ($n = 150$), and microscopic scoring was used to assess subdural hemorrhage (SDH) and parenchymal hemorrhage within specific brain regions that are responsible for consciousness and vital function ($n = 32$). The Zephyr-E caused immediate, sustained insensibility until death in 98.6% of pigs. On average, clonic convulsions (CC) ceased in 82.2 s (±3.4 SE), brain death was achieved in 144.9 s (±5.4 SE), and cardiac arrest occurred in 226.5 s (±8.7 SE). Time of brain death and cardiac arrest differed significantly among stock people ($P = 0.0225$ and $P = 0.0369$). Age was positively related to the duration of CC ($P = 0.0092$), time of brain death ($P = 0.0025$), and cardiac arrest ($P = 0.0068$) with shorter durations seen in younger pigs. Average FD was 8.3 mm (±1.0 SE). Macroscopic scores were significantly different among weight classes for subcutaneous ($P = 0.0402$) and subdural-ventral ($P = 0.0037$) hemorrhage with the lowest severity hemorrhage found in the 9-kg weight category. Microscopic scores differed among brain sections ($P = 0.0070$) for SDH with lower scores found in the brainstem compared to the cerebral cortex and midbrain. Parenchymal hemorrhage differed among brain sections ($P = 0.0052$) and weight categories ($P = 0.0128$) with the lowest scores in the midbrain and brainstem and the 7- and 9-kg weight categories. The Zephyr-E was highly effective for the euthanasia of pigs up to 9 kg (49 d) based on immediate insensibility sustained until death. Postmortem results confirmed that severe skull fracture and widespread brain hemorrhage were caused by the Zephyr-E nonpenetrating captive bolt.

**Key words:** animal welfare, captive bolt, euthanasia, insensibility, nursery, pig



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## INTRODUCTION

For suckling pigs, the transition into the nursery stage is a stressful time. During this period, weak, injured, or diseased pigs are at a significantly higher risk of mortality (Hameister et al., 2010). Euthanasia of compromised nursery pigs has been shown to improve group welfare and provide economic benefits to the producer (Morrow et al., 2006). Physical methods of euthanasia, such as manual blunt force trauma (**BFT**), are the most practical for the farrowing house and nursery (Matthis, 2004). However, pigs grow rapidly from 1.5 kg at birth

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to 13 kg by 7 wk of age (Schinckel et al., 2003) making euthanasia by physical methods during the nursery phase uniquely difficult because of the size of the pig (Matthis, 2004). Moreover, BFT is not recommended for use on pigs >5.5 kg (AASV and NPB, 2009), and the American Veterinary Medical Association encourages "those using manually applied blunt force trauma…to actively search for alternatives to ensure that criteria for euthanasia can be consistently met" (AVMA, 2013, p. 61–62).

Captive bolt guns provide a mechanical approach, reducing the reliance on stock person strength. However, in a survey of over 300 stock people, Matthis (2004) reported that over 20% of respondents were concerned for operator safety with captive bolt guns and over 40% found the open wound left by the captive bolt unsightly or upsetting. Recently, a nonpenetrating captive bolt (**NPCB**), Zephyr-E, was shown to be highly effective for euthanasia of neonatal piglets and received positive feedback in terms of esthetics and ease of use from stock people on participating farms (Casey-Trott et al., 2013).

The objective of this study was to determine the effectiveness of the Zephyr-E for euthanasia of suckling and weaned pigs from 3 to 9 kg. Time of insensibility and cardiac arrest were outcome measures used to determine whether the technique was effective and humane. Postmortem assessments were used to quantify the degree of traumatic brain injury (**TBI**).

## METHODS

All procedures were approved by the University of Guelph Animal Care Committee as well as by the University of Saskatchewan's Committee on Animal Care and Supply and the Animal Research Ethics Board. A total of 150 low-viability pigs ranging from 2.5 to 10.2 kg and all less than 50 d of age were used in the study. All pigs were of common commercial stock and were identified for euthanasia by farm personnel because they were either compromised by poor health status or low birth weight and required euthanasia according to the farm animal care protocols. The methods described for the current study followed the methodology of a previous study, which tested the effectiveness of the Zephyr-E on neonatal pigs (see Casey-Trott et al., 2013).

### *Euthanasia Device and Procedures*

The Zephyr-E (Fig. 1) is a pneumatic nail gun (NS 100A 1/4" Narrow Crown Stapler; Porter Cable Corporation, Jackson, TN) that is modified to hold a conical nylon bolt head (diameter: 2.5 cm, and length: 3.8 cm) attached to a cylindrical bolt (diameter: 0.8 cm; Erasmus et al., 2010a). The nylon bolt head is recessed 3.3 cm into a metal barrel of the gun. When fully extended, the nylon bolt head protrudes 1.9 cm from the end of the gun barrel. The Zephyr-E attaches to a standard air compressor and is applied with an airline pressure of 794 to 827 kPa (115–120 pounds per square inch). The Zephyr-E is lightweight (1.02 kg) and allows for multiple applications (shots) in rapid succession by repeatedly depressing the trigger without reloading cartridges.


**Figure 1.** Photo of the Zephyr-E equipment in the fired position with bolt head protruding (left) and the unfired position attached to a standard air compressor (right). See online version for figure in color.

Data were collected on 2 university research farms and 4 commercial farrowing and nursery units in Ontario and Saskatchewan. Fifteen stock people who were routinely responsible for performing euthanasia at the farms were trained to use the Zephyr-E. Stock people 1 through 4 were from farm 1, stock people 5 through 7 were from farm 2, stock person 8 was from farm 3, and stock people 10 through 13 were from farm 4. Farms 1 through 3 were all in southern Ontario and farm 4 was in Saskatchewan. Stock person 9 was from the research station in Ontario and stock people 14 and 15 were from the research station in Saskatchewan. All Saskatchewan trials were run by a research technician from the University of Saskatchewan who was trained to use the Zephyr-E and conduct antemortem and postmortem data collection.

Pigs were selected from 4 weight classes: 3 kg = 2.5 to 3.9 kg ($n$ = 55), 5 kg = 4.0 to 5.9 kg ($n$ = 45), 7 kg = 6.0 to 7.9 kg ($n$ = 25), and 9 kg = 8.0 to 10.2 kg ($n$ = 25). Due to the reduced availability of compromised 7- to 9-kg pigs, power analyses were conducted to determine the minimum sample size to detect failure to cause cardiac arrest in pigs within this weight range. Based on the results reported for neonatal piglets euthanized by the Zephyr-E (Casey-Trott et al., 2013), it was determined that 144 pigs in total were required to determine whether the technique was >96% effective in pigs 3 to 9 kg ($\alpha$ = 0.05).

Each pig was restrained in a portable animal restraint sling (Lomir Biomedical Inc., Notre-Dame-de-

**Table 1.** Macroscopic scoring system[1]

| | Fracture score description | Hemorrhage score[2] description | | |
|---|---|---|---|---|
| Score | SK[3] | SC | SDD | SDV |
| 0 | No fractures, intact skull | No hemorrhage | | |
| 1 | Hairline fractures, no separation of bone | Less than 25% of surface area covered | | |
| 2 | One to two complete fully separated fractures or single depressed fracture | 26–50% of surface area covered | | |
| 3 | More than just a single depressed fracture, 3 to 5 complete fractures | 51–75% coverage | | |
| 4 | More than 5 complete fractures, fully fragmented skull | 76–99% coverage | | |
| 5 | N/A[4] | Complete coverage | | |

[1]Adapted from Erasmus et al. (2010b).

[2]SC = subcutaneous; SDD = subdural-dorsal; SDV = subdural-ventral.

[3]SK = skull fracture.

[4]N/A = Not applicable- Skull fracture was scored on a scale from 0-4.

l'Île-Perrot, QC, Canada). The pig's legs were placed through 4 holes in the canvas sling. The head of the pig was supported by the broad, firm surface of the canvas sling to ensure a consistent direction of force transfer. The stock person placed 1 hand over the shoulders of the pig and operated the Zephyr-E with the other hand. As described in Casey-Trott et al. (2013), 2 shots were administered rapid fire on the frontal bone (AASV and NPB, 2009); however, an additional shot behind the ear could not be performed for safety reasons due to the immediate onset of rigorous convulsions.

### *Antemortem Data Collection*

Immediately after Zephyr-E application, pigs were assessed by the researchers for signs of sensibility using brainstem reflexes: corneal reflex, pupillary light reflex, jaw tone, and response to nose prick (Casey-Trott et al., 2013).

Onset and duration of clonic and tonic neuromuscular leg spasms (convulsions) and presence of respiration were monitored by visual assessment. The duration of clonic convulsions (**CC**) began at the onset of leg paddling and ended when the transition into the tonic convulsion phase began. Tonic convulsions were defined as rigid extension of the limbs. The total convulsion duration (**TC**) combined the duration of clonic and tonic convulsions to the point at which the pig became completely limp and motionless. Presence and duration of heartbeat (**HB**) were determined by palpation or auscultation. Cardiac arrest was determined to have occurred when no discernible HB could be found by auscultation or palpation.

Reflexes were repeatedly checked in the order listed above every 15 s along with continuous monitoring of the convulsive stages until the animal was considered brain dead or for a maximum observation period of 15 min. An animal was considered to be brain dead when all reflexes, convulsions, and breathing were absent.

The research protocol required that for any pigs exhibiting signs of returning to consciousness, the Zephyr-E was immediately reapplied. If the method did not successfully induce sustained unconsciousness or if cardiac arrest was not achieved within 15 min, an alternative euthanasia technique was used. Anesthetic overdose (pentobarbital sodium [340 mg/mL] 0.3 mL/kg Intracardiac; Euthansol; Schering-Plough [now Merck Animal Health], Kirkland, QC, Canada) was used as a secondary method on the research farm and BFT was used on the commercial farm.

### *Postmortem Data Collection*

A subset of the pigs from each weight class ($n$ = 24; 3 kg: $n$ = 6; 5 kg: $n$ = 5; 7 kg: $n$ = 7; and 9 kg: $n$ = 6) that were euthanized in Ontario were scanned by computed tomography (**CT**) within 3 h of euthanasia by technicians at the Department of Clinical Studies, Ontario Veterinary College (Guelph, Ontario). As described in Casey-Trott et al. (2013), CT scans were completed and later evaluated by a veterinary radiologist (S.G. Nykamp) for hemorrhage severity (0 = no hemorrhage, 1 = mild, 2 = moderate, and 3 = severe) and fracture displacement (**FD**). Fracture displacement was recorded as the distance (mm) between the normal position of the cortical bone and where the cortical bone had been displaced. Skull thickness (mm) was also measured.

All 150 pigs were scored macroscopically during gross dissection for skull fracture (**SK**) and subcutaneous (**SC**), subdural-dorsal (**SDD**), and subdural-ventral (**SDV**) hemorrhage as described in Casey-Trott et al. (2013). Macroscopic scoring was based on a 0 to 5 point scale (Table 1), adapted from Erasmus et al. (2010b). Before dissection, each pig was weighed (kg) and crown to rump length was recorded (cm) to calculate body mass index (**BMI** = mass (kg)/(length)$^2$ (m)$^2$).

After gross macroscopic evaluation, brains of the 32 pigs (3 kg: $n$ = 8; 5 kg: $n$ = 8; 7 kg: $n$ = 8; and 9 kg: $n$ = 8), including 22 brains that underwent CT scans, were removed and placed in 10% buffered formalin for at least 7 d to prepare for histologic analyses. Once fixed, 3 sections were trimmed from the right hemisphere of each brain from the following areas: cerebral cortex, midbrain and thalamus, and brainstem. Brain sections were scored without knowledge of weight category by a veterinary pathologist (P.V. Turner) to determine the degree and location of subdural hemorrhage (**SDH**) and parenchymal hemorrhage (**PH**). Scores were based on the relative area of brain showing hemorrhage in proportion to total area of the brain on the entire slide (Table 2). Preparation and scoring details are described in Casey-Trott et al. (2013).

**Table 2.** Microscopic scoring system[1]

| Score | Percent of brain section affected[2] |
|---|---|
| 0 | None (0%) |
| 1 | Minimal (<5%) |
| 2 | Mild (<10%) |
| 3 | Moderate (<30%) |
| 4 | Marked (>30%) |

[1]Adapted from Erasmus et al. (2010b).

[2] Subdural and parenchymal hemorrhage were determined by the percent of hemorrhage within each location for each section.

### Statistical Analyses

All statistical analyses were computed in SAS 9.2 (SAS Inst. Inc., Cary, NC). Student's *t* tests were used to test for differences between the convulsion and HB duration data sets from Ontario and Saskatchewan. No significant differences were found; therefore, all data was compiled and run as a single data set. Mixed model ANOVA were used to test for overall mean differences among stock people for durations of CC, TC, and HB with stock person as a fixed effect nested within farm. To account for farm differences, farm was included as a random effect. Several studies have reported a maturation-dependent response to brain trauma in pigs (Duhaime et al., 2000; Ibrahim et al., 2010; therefore, an age × BW interaction was included in the model as a covariate. Duration of CC, TC, and HB were dependent variables. Body mass index has also been shown to correlate with higher rates of stillbirths and postnatal mortality (Baxter et al., 2008) and had a significant negative relationship with the duration of CC (Casey-Trott et al., 2013). To account for growth variation and morbidity effects, the mixed model of stock person nested within farm was repeated using an age × BMI interaction. Data were assessed for normality using Shapiro-Wilk analyses. The duration of CC, TC, and HB were log-transformed to normalize the data. Raw means and SE are presented in the results. Log-transformed means and SE for stock person mean duration of TC and HB as well as the log-transformed durations of CC, TC, and HB are reported in the results.

Regression analyses were conducted to test for linear relationships between FD and age, weight, and BMI as single variables as well as for any relationships between FD and age × weight and age × BMI interactions. Regression analyses were also conducted to test for linear relationships between skull thickness and age, weight, and BMI as single variables as well as for any relationships between FD and age × weight and age × BMI interactions.

Hemorrhage data were rank transformed (Akritas, 1990) using proc RANK. A 1-way ANOVA was run on the ranked data to test the effects of brain section on hemorrhage scores for hemorrhage severity; SK; and SC, SDD, and SDV hemorrhage and a 2-way ANOVA was run on the ranked microscopic data to test the effects of brain section and weight class in SDH and PH.

Statistical significance was defined as $P < 0.05$.

## RESULTS

### Antemortem Sensibility Assessment

Immediate, sustained insensibility until cardiac arrest was achieved in 98.6% of pigs euthanized using the Zephyr-E. Of the remaining 1.4% (2 pigs), 1 pig exhibited a blink in response to the corneal reflex test and the Zephyr-E was immediately reapplied resulting in immediate loss of the reflex. The other pig was rendered immediately unconscious but began to transition from gasping to rhythmic breathing. After an extra shot behind each ear, all gasping stopped. Death was achieved without a secondary step in 99.3% of pigs (149 pigs). Blunt force trauma was used as an alternative method for 1 pig that was rendered unconscious but exhibited an atypical convulsion pattern with sporadic movements.

The average duration of CC was 82.2 s (±3.4 SE). The average duration of TC was 144.9 s (±5.4 SE). The average HB duration was 226.5 s (±8.7 SE). Durations of CC, TC, and HB were classified into 1-min intervals and plotted across time to show the cumulative percentage of pigs ceasing CC, TC, or HB at any 1 time point (Fig. 2).

With an age × BW interaction as the covariate, stock person had a significant effect on TC ($P = 0.0225$; Fig. 3). Stock person 4 had significantly longer durations than 10 other stock people. Age had a significant effect on the duration of CC ($P = 0.0092$, $R^2 = 0.1143$), TC ($P = 0.0025$, $R^2 = 0.0816$), and HB ($P = 0.0068$, $R^2 = 0.1031$), each of which increased as age increased. With an age × BMI interaction as the covariate, stock person had a significant effect on the duration of HB ($P = 0.0369$). Stock person 1 had significantly shorter durations than 11 other stock people within the group (Fig. 4). Body mass index and the age × BMI interaction had a significant effect on the duration of CC: $P = 0.0076$ ($R^2 = 0.0358$) and $P = 0.0171$, respectively. However, based on the $R^2$ values, age and BMI accounted for only a small degree of variation. The duration of CC decreased as BMI increased. Age had no significant effect on CC ($P = 0.0694$) but showed a positive trend, with the duration of CC increasing with age.

### Postmortem Assessment

Based on CT results, the mean hemorrhage severity score was 0.88 (±0.1 SE). Hemorrhage severity was not significantly different among weight categories ($P = 0.2362$). Mean FD was 8.5 mm (±0.09 SE). All pigs had

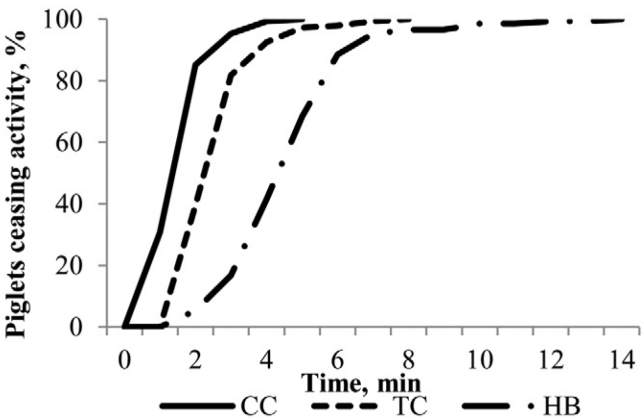


**Figure 2.** Cumulative percentage of pigs ceasing clonic convulsions (CC), total convulsion duration (TC), and heartbeat (HB) across time. Time point 0 indicates the time immediately following Zephyr-E application. Two pigs required a secondary step and were not included in the duration results ($N = 148$). Ninety-five percent of pigs ceased CC in <3 min with all pigs ceasing CC in <5 min. Ninety-three percent of pigs achieved brain death in <4 min with all remaining pigs ceasing TC in <8 min. Ninety-five percent of pigs achieved cardiac arrest in <7 min with all pigs achieving cardiac arrest in <14 min.

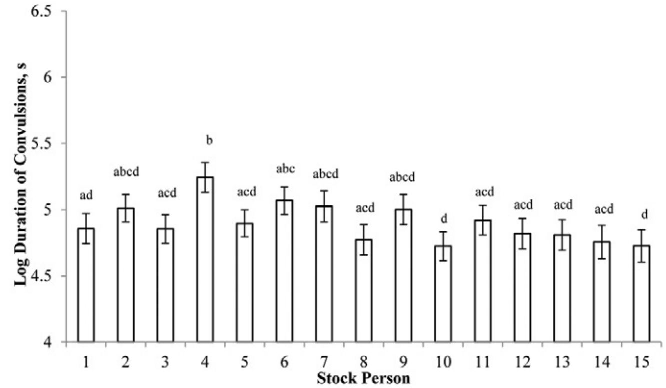


**Figure 3.** Stock person had a significant effect on the total convulsion duration (TC; $P = 0.0225$). $^{a–d}$Means without a common superscript differ ($P < 0.05$). Raw mean duration of TC: 144.9 sec (±5.4 SE). Stock people 1 through 4 were from commercial farm 1. Stock people 5 through 7 were from commercial farm 2. Stock person 8 was from commercial farm 3 and stock people 10 through 13 were from commercial farm 4. Stock person 9 was from the Ontario research station and stock people 14 and 15 were from the Saskatchewan research station.

SK; however, 1 pig (8.6 kg) had fractures present without displacement. Fracture displacement showed no linear relationship with age ($P = 0.6210$), weight ($P = 0.4196$), BMI ($P = 0.8637$), age × weight ($P = 0.4730$), or age × BMI ($P = 0.5661$). Skull thickness had a significant positive linear relationship with BMI ($P = 0.0356$, $R^2 = 0.2024$). The average skull thickness for each weight class was as follows: 3 kg: 2.9 mm (±0.04 SE); 5 kg: 3.6 mm (±0.06 SE); 7 kg: 3.8 mm (±0.03 SE); and 9 kg: 3.9 mm (±0.01 SE).

Macroscopic scores indicated moderate to severe hemorrhage in ≥85% of pigs in each weight category. Subcutaneous and SDV hemorrhage scores were significantly different among weight categories ($P = 0.0402$ and $P = 0.0037$; Table 3). Moderate to severe SDV hemorrhage was reported in ≥60% of pigs from the 3-, 5-, and 7-kg weight categories, whereas SDV hemorrhage in the 9-kg category was predominantly minimal to mild.

Microscopic analyses confirmed the presence of SDH and PH in all pigs. There were significant differences in SDH scores among brain sections ($P = 0.0070$) but not among weight categories ($P = 0.3592$; Table 4). Significant differences in PH scores were present across brain sections ($P = 0.0052$) as well as across weight categories ($P = 0.0128$; Table 5).

## DISCUSSION

The Zephyr-E consistently provided a humane death for pigs between 3 and 9 kg by causing immediate, sustained insensibility until cardiac arrest. Immediate insensibility was induced in 98.6% of pigs with only 2 pigs showing signs of return to consciousness. Similar results were reported when the Zephyr-E was used on neonatal pigs, where 100% of the pigs were determined immediately insensible by monitoring brainstem reflexes (Casey-Trott et al., 2013). The effectiveness of the Zephyr-E was also comparable to the results of other methods tested on pigs greater than 5 kg: 100% for NPCB, 93 to 99% for free bullet methods, 98% for anesthetic overdose, and 97% for BFT (Whiting et al., 2011). It is important to note that the methods used for detecting return to consciousness in the study reported here were more comprehensive than those used by Whiting et al. (2011) in which brainstem reflexes were not monitored. Even with more precise measures of sensibility, the Zephyr-E only required repeated application for 1.4% of pigs, whereas all the techniques reported by Whiting et al. (2011), except NPCB, required repeated applications to ensure insensibility in 5 to 24% of pigs.

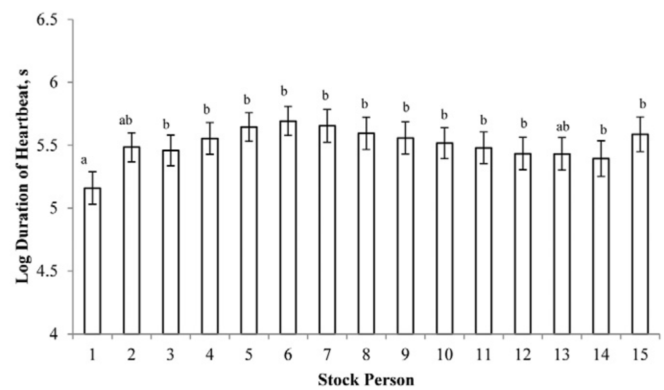


**Figure 4.** Stock person had a significant effect on time to cardiac arrest ($P = 0.0369$). $^{a,b}$Means without a common superscript differ ($P < 0.05$). Raw mean duration of heartbeat: 226.5 sec (±8.7 SE). Stock people 1 through 4 were from commercial farm 1. Stock people 5 through 7 were from commercial farm 2. Stock person 8 was from commercial farm 3 and stock people 10 through 13 were from commercial farm 4. Stock person 9 was from the Ontario research station and stock people 14 and 15 were from the Saskatchewan research station.

**Table 3.** Macroscopic scores across weight categories

| Weight category,[1] kg | Macroscopic score[2] (±SE) | | | |
|---|---|---|---|---|
| | SC | SDD | SDV | SK |
| 3 | 3.7 (±0.1)[a] | 3.3 (±0.1) | 3.2 (±0.1)[a] | 2.5 (±0.1) |
| 5 | 3.4 (±0.2)[ab] | 3.5 (±0.1) | 3.1 (±0.1)[a] | 2.3 (±0.1) |
| 7 | 3.3 (±0.2)[ab] | 3.4 (±0.2) | 2.7 (±0.2)[ab] | 2.5 (±0.1) |
| 9 | 2.9 (±0.2)[b] | 3.6 (±0.2) | 2.3 (±0.2)[b] | 2.2 (±0.1) |

[a,b]Within a column, means without a common superscript differ ($P < 0.05$).

[1]Weight category ranges: 3 kg (2.5–3.9 kg; $n = 55$), 5 kg (4.0–5.9 kg; $n = 45$), 7 kg (6.0–7.9 kg; $n = 25$), and 9 kg (8.0–10.2 kg; $n = 25$).

[2]SC = subcutaneous; SDD = subdural-dorsal; SDV = subdural-ventral; SK = skull fracture.

**Table 4.** Microscopic subdural hemorrhage (SDH) scores (±SE) across weight categories and brain sections

| Weight category, kg | Brain section[1] | | | Weight category means (±SE) |
|---|---|---|---|---|
| | Cerebral cortex | Midbrain | Brainstem | |
| 3 | 3.0 ± 0.2 | 2.8 ± 0.2 | 2.4 ± 0.4 | 2.7 ± 0.2 |
| 5 | 3.2 ± 0.2 | 3.4 ± 0.4 | 2.1 ± 0.2 | 2.9 ± 0.2 |
| 7 | 2.9 ± 0.2 | 2.9 ± 0.4 | 2.5 ± 0.6 | 2.7 ± 0.3 |
| 9 | 2.5 ± 0.2 | 2.7 ± 0.4 | 2.4 ± 0.4 | 2.5 ± 0.3 |
| Brain section means (±SE) | 2.9 ± 0.1[a] | 2.9 ± 0.2[a] | 2.4 ± 0.2[b] | |

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Each brain was divided into 3 coronal sections. $n = 32$ brains; 96 sections total for SDH (3 kg, $n = 8$; 5 kg, $n = 8$; 7 kg, $n = 8$; and 9 kg, $n = 8$).

When considering the 2 pigs that required repeat application in the current study, 1 pig (2.7 kg) had a blink response clearly following a misfire related to operator error. The stock person used their nondominant hand to handle the Zephyr-E and the barrel was not placed flush with the forehead of the pig. With an additional, properly placed shot, all reflexes were immediately absent and the pig rapidly progressed to death without further intervention. This emphasizes the importance of proper training and clear instruction and oversight until the operator is comfortable with the new technique. The head of the pig must be supported by a broad, firm surface, such as a restraint device, countertop, or solid floor. The second pig (8.2 kg) showed no response to any brainstem reflexes but exhibited sporadic gasps that appeared to be transitioning into rhythmic breathing. Although gasps are considered involuntary movements that can occur during unconsciousness (Wijdicks, 1995), the return to rhythmic breathing was a concern because breathing is one of the earliest signs of returning to consciousness for swine (Anil, 1991). The Zephyr-E was immediately reapplied behind the ear, ending all gasping. The shot behind the ear is intended to target the brainstem, which is responsible for respiratory and cardiac regulation (Shaw, 2002). Therefore, an additional shot behind the ear is recommended for pigs exhibiting any gasping behavior. It is important to note that this particular pig was a cryptorchid, whereas all other males pigs used in this study were castrated. It has been suggested that an increase in circulating sex steroids may provide protection against neuronal damage resulting from a TBI (Shear et al., 2002; Hurn et al., 2005). Unfortunately, the sex of the pigs was not recorded in this trial. The sex differences of this particular pig were recorded because cryptorchidism was the reason for euthanasia of an otherwise healthy pig. Overall, the vast majority of pigs were rendered immediately and irreversibly insensible.

By achieving rapid insensibility and brain death, the Zephyr-E minimized pain and distress suffered by the animal and prevented the regain of vital function, effectively accomplishing some of the most important criteria for assessing euthanasia methods as described by the American Veterinary Medical Association (2013) in a single step. According to the American Association of Swine Veterinarians and the National Pork Board (2009), a single step method is a process that causes irreversible insensibility and leads to death, whereas a 2-step method stuns the animal with 1 technique but requires an adjunctive method to cause death. Since the Zephyr-E does not require calibrating or reloading, the 2 shots can be fired within milliseconds of each other, with the second shot in place as a precaution to ensure a substantial blow to the head. The results reported here confirm that the 2 rapid-fire shots caused immediate, sustained insensibility until death in 99.3% of pigs without a secondary step.

Although the ideal method to confirm brain death in animals is an isoelectric electroencephalogram (**EEG**), the location of NPCB application is not conducive for use of EEG. Instead, clinical criteria including loss of brainstem reflexes and motor responses to pain in combination with apnea and the cessation of movement have been used to estimate the time of brain death (Casey-Trott et al., 2013). Similar techniques such as apnea in the absence of brainstem reflexes and the cessation of convulsive activity are used to diagnose brain death in humans (Wijdicks, 1995, 2001; Hills, 2010) and poultry (Dawson et al., 2007, 2009; Turner et al., 2012), respectively. Turner et al. (2012) also reported the presence of a heartbeat in poultry several minutes after brain death, as confirmed by use of an electrocardiogram and EEG. A fluctuating heartbeat following brain death is not uncommon in humans (Conci et al., 2001), with human infant hearts capable of beating for greater than 20 min in an anaerobic environment (Aroni et al., 2012). This coincides with the results in the present study as the majority of pigs, although completely limp and motionless with fixed and fully dilated pupils, briefly retained a faint, irregular heartbeat. This continuation of a heartbeat is a result of glycogen supplies within the cardiac muscle (Aroni et al., 2012). As expected, these pigs had clearly succumbed to irreversible TBI and progressed to cardiac arrest in a timely manner. Even with a conservative approach for recording heartbeat, cardiac

**Table 5.** Microscopic parenchymal hemorrhage[1] (PH) across weight categories and brain sections

| Weight category, kg | Brain section[2] Cerebral cortex | Midbrain | Brainstem | Weight category means (±SE) |
|---|---|---|---|---|
| 3 | 2.1 ± 0.4 | 1.6 ± 0.4 | 1.6 ± 0.4 | 1.8 ± 0.2[xz] |
| 5 | 2.6 ± 0.4 | 1.5 ± 0.4 | 1.0 ± 0 | 1.9 ± 0.2[x] |
| 7 | 1.7 ± 0.2 | 0.8 ± 0.2 | 1.0 ± 0 | 1.1 ± 0.2[y] |
| 9 | 1.4 ± 0.2 | 1.4 ± 0.3 | 0.9 ± 0.2 | 1.2 ± 0.2[yz] |
| Brain section means (±SE) | 1.9 ± 0.2[a] | 1.3 ± 0.1[b] | 1.3 ± 0[b] | |

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

[x–z]Within a column, means without a common superscript differ ($P < 0.05$).

[1]Mean microscopic score (±SE): PH mean (± SE).

[2]Each brain was divided into 3 coronal sections. $N$ = 32 brains and 96 sections total for PH (3 kg, $n$ = 8; 5 kg, $n$ = 8; 7 kg, $n$ = 8; and 9 kg, $n$ = 8).

arrest occurred in nearly half the time of that reported for neonates euthanized by NPCB (Casey-Trott et al., 2013) and for pigs <8 kg euthanized by manual BFT (Chevillon et al., 2004) and at a similar time for pigs >8 kg killed with penetrating captive bolt (Chevillon et al., 2004).

An ethical decision was made to use an alternative method before cardiac arrest for 1 (7.5 kg) pig following application of the Zephyr-E. Although this pig was rendered insensible and progressed normally through the convulsive phase, spontaneous movements were triggered when the researched palpated the animal to check for a heartbeat. This type of sporadic movement has also been reported in neonatal pigs (Casey-Trott et al., 2013) and is postulated to be similar to the spontaneous movements occasionally seen in humans after confirmed brain death or severe brain trauma (Ropper, 1984). Spontaneous body movements, such as head turning, flexion at the waist, and arm raising, can occur when human patients determined to be brain dead are handled as the manipulation may initiate movements that are generated by spinal reflexes (Wijdicks, 2001). Humans and other animals have also been reported to show spontaneous, repetitive limb "stepping movements" following extensive brainstem pathologies and when in a comatose state (Hanna and Frank, 1995; Lee et al., 2005). Overall, the success of the Zephyr-E in this study is in stark contrast to the results of Finnie et al. (2003), who reported that all pigs survived the use of a NPCB. However, a temporal position was used by Finnie et al. (2003), confirming the importance of NPCB placement on the forehead of swine. Forehead placement was also found to cause immediate insensibility and death in weaned pigs using the Cash Euthanizer NPCB (Woods, 2012).

In terms of the visual esthetics of technique, the convulsive stage, arguably the most disturbing period to an unfamiliar audience, was actually shorter for weaned pigs than the durations reported for neonates (Casey-Trott et al., 2013). Conversely, Sadler et al. (2014) observed that neonates succumbed faster than weaned pigs when using inhalant euthanasia techniques with carbon dioxide and argon gases. This contrasting result may be related to the differences in the mode of action between physical and gaseous euthanasia techniques, with physical methods causing direct destruction of neuronal tissues and gaseous methods causing hypoxic conditions in the brain leading to tissue death (AVMA, 2013). Paddling movements ended within 2 min for 85% of pigs and within 5 min for 100% of pigs observed. These grand mal convulsive movements are involuntary neuromuscular responses indicative of epileptiform brain activity following severe concussion (Shaw, 2002) and during electrical stunning in pigs (McKinstry and Anil, 2004). Varying degrees of these convulsive movements have been reported following application of all available euthanasia techniques for pigs including BFT and NPCB (Chevillon et al., 2004; Widowski et al., 2008; Woods, 2012) and $CO_2$ (Raj and Gregory, 1996; Sutherland, 2010; Sadler et al., 2014). The convulsive period was similar to the 1 to 1.5 min typically seen with BFT (Chevillon et al., 2004; Widowski et al., 2008) and shorter than other NPCB devices falling within the range or 1 to 4 min reported by Woods (2012).

From the postmortem assessment, it is clear that the application of the Zephyr-E to the frontal bone position (AASV and NPB, 2009) induced severe SK and widespread brain hemorrhage. The cerebral cortex, a region responsible for sensory processing and signaling to the brainstem (Shaw, 2002; Gaetz, 2004), suffered severe damage inflicted by SK pieces embedded in the brain tissue, likely contributing to interruptions in cerebral blood flow (Nedd et al., 1993). Severe hemorrhage was also consistently found and may have led to increased intracranial pressure, a life-threatening condition (Young and Destian, 2002). Not only was a massive localized injury found at the site of impact, but hemorrhage was present throughout the brain to a lesser degree. The widespread damage observed on the ventral surface of the brain during macroscopic assessment as well as within the brain sections in the microscopic assessment is likely the combined result of a "coup/contrecoup" type injury caused by the rapid impact forces jolting the brain within the cranium (Gaetz, 2004) and a diffuse injury caused by a change in intracranial pressure from both the initial displacement of the brain as well as from secondary edema from the injury (Madsen, 1990). Traumatic brain injury of this magnitude likely caused irreversible destruction to regions of the brain responsible for aspects of consciousness and vital function.

Although application of the Zephyr-E was highly effective for euthanasia of pigs in all of the weight categories tested in this study, there were some effects of BW, BMI, and age on the antemortem and postmortem variables. Pig age had the most widespread effects on both antemortem and postmortem variables. This is likely related to the maturation-dependent response to TBI reported in several studies in which pigs have been used as animal models for TBI in human infants (Duhaime et al., 2000; Durham and Duhaime, 2007; Missios et al., 2009; Ibrahim et al., 2010). In the present study, when BW was accounted for, the durations of CC, TC, and HB all increased as age increased.

The interaction between BMI and age illustrated the complexity of the mechanisms of TBI on death in pigs as the interaction had a significant effect on CC. Older pigs with a low BMI consistently had a longer duration of CC in comparison to older pigs with a higher BMI. There was also an inverse relationship between BMI and the duration of CC, a result previously reported in neonatal pigs (Casey-Trott et al., 2013). Perhaps the differences can be explained in part by the positive relationship between BMI and skull thickness, in that pigs with low BMI had thinner skulls, potentially reducing the severity of concussion or brain injury. It is also possible that the low BMI pigs had metabolic or physiologic differences as suggested by Baxter et al. (2008), thereby altering the mechanism and severity of TBI, causing older pigs with a low BMI to be mildly resistant to the TBI inflicted by the Zephyr-E.

Overall, the extent of hemorrhage throughout the brain was less severe in the heaviest weight categories, whereas FD was noticeably more severe in the heaviest weight categories. The increased FD is likely due to thickening and calcification of the skull making it less pliable and more subject to fracture. Although the mechanism is not completely understood, it is clear from the literature that the neonatal brain is more resistant to rotation- (Ibrahim et al., 2010) and impact-related TBI (Duhaime et al., 2000) than the brain of an older pig. The reduction in overall hemorrhage seen in the larger pigs was initially thought to be indicative of less severe and therefore less effective TBI induced by the Zephyr-E; however, it is also possible that the more severe hemorrhage reported in neonates (Casey-Trott et al., 2013) and 3- to 5-kg weight categories could be explained by the theory of "neoprotection." Durham and Duhaime (2007) postulate that severe SDH alone is not sufficient to overwhelm the neonatal brain, but it is instead the combination of severe hematoma with apnea and seizures that negate the inherent protection in neonates. Furthermore, pigs older than 4 wk of age were much more susceptible to both subdural hematoma (Duhaime et al., 2000; Durham and Duhaime, 2007) and shear strain of brain tissue (Ibrahim et al., 2010). Human infants also exhibit a similar form of neoprotection. Perinatal infants are capable of maintaining a prolonged heartbeat in an anaerobic environment; however, this characteristic is quickly lost with age, increasing the risk of brain damage and death resulting from hypoxia in older infants (Aroni et al., 2012). This fragility may be why the older, heavier pigs succumbed to their injuries faster than their neonatal counterparts before extensive PH occurred.

The Zephyr-E provided a humane death by reliably causing severe brain trauma with minimal variation among those applying it. The mean variation in the present study was limited to 2 individuals. The pigs euthanized by stock person 4 had a significantly higher average TC than 10 other stock people in this study. This variation did not reduce the effectiveness of the technique and may have been related to the fact that the 10 pigs euthanized by stock person 4 were the only pigs in this study that were still suckling and had not yet been weaned. Mean HB also varied among stock people with the pigs euthanized by stock person 1 having a significantly shorter mean HB than the pigs euthanized by 11 other stock people. Stock person 1 was later identified as the researcher who had more experience with the equipment due to involvement in previous trials. Although this may be considered a confounding factor, it may also indicate that the effectiveness of the technique can be improved with experience.

In terms of esthetics, a bruise was present on the forehead of the pigs, similar to the physical trauma reported by Casey-Trott et al. (2013). Nasal bleeding was noted in approximately 20% of pigs, but skin puncture was rare (<5%) and bleeding through the ear was not observed.

Based on the outcome measures indicating immediate insensibility, timely death, and irreversible TBI, it can be concluded that the Zephyr-E reliably induces a humane end for pigs from 3 to 9 kg as a single-step euthanasia method. Not only does this confirm the recommendations by the National Pork Board, American Association of Swine Veterinarians (AASV and NPB, 2009), and World Organisation for Animal Health (2011) that a NPCB is an acceptable method of euthanasia for pigs greater than 5.5 kg, but it also suggests that the method can be used without a secondary step.

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