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Effectiveness of a nonpenetrating captive bolt for euthanasia of piglets less than 3 d of age¹

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ABSTRACT: The objective of this study was to determine the effectiveness of a nonpenetrating captive bolt (NPCB), the Zephyr-Euthanasia (Zephyr-E), for euthanasia of neonatal piglets < 72 h of age using signs of insensibility and death, as well as postmortem assessment of traumatic brain injury (TBI). The Zephyr-E was used by 10 stock people to euthanize 100 low viability neonatal piglets from 3 commercial farrowing units and 1 research farm. Brainstem reflexes, convulsions, and heartbeat were used to assess insensibility, time of brain death, and cardiac arrest after Zephyr-E application. Hemorrhage severity and skull fracture displacement (FD) were quantified from computed tomography scans ($n = 10$), macroscopic scoring was used to assess brain hemorrhage and skull fracture (SK) severity ($n = 100$), and microscopic scoring was used to assess subdural (SDH) and parenchymal (PH) hemorrhage within specific brain regions that are responsible for consciousness and vital function ($n = 10$). All 100 piglets were rendered immediately insensible

without return to sensibility. On average, clonic convulsions (CC) ceased in 101 s (± 7.4 SE), brain death was achieved in 229 s (± 9.18 SE), and cardiac arrest occurred in 420 s (± 13.57 SE). Time of cardiac arrest differed significantly among stock people when either body weight (BW: $P = 0.0053$) or body mass index (BMI: $P = 0.0059$) was used as a covariate. The BMI was inversely related to the duration of CC ($P = 0.0227$). Moderate to severe hemorrhage severity was reported in 9 of 10 piglets. There was no relationship between FD and BW ($P = 0.8408$) or BMI ($P = 0.6439$). Macroscopic analyses indicated moderate to severe hemorrhage and SK in all piglets. No differences were found among brain sections for SDH ($P = 0.2302$); PH was greater in the cerebral cortex than in the midbrain and brainstem ($P = 0.0328$). The Zephyr-E NPCB reliably caused immediate, sustained insensibility followed by death in neonatal piglets. Postmortem assessment confirmed that application of the Zephyr-E caused widespread, irreversible brain damage.

Key words: animal welfare, captive bolt, euthanasia, insensibility, neonate, piglet

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INTRODUCTION

Few researchers have evaluated the animal welfare impact of techniques considered to be acceptable

for on-farm euthanasia and culling for disease control purposes (Irwin, 2010). Physical methods that involve impact to the skull with a solid object are the most practical techniques for euthanasia of piglets on farms (Millman, 2010). By causing severe damage to the central nervous system, these methods disrupt sensory processing and prevent adequate oxygen flow to vital regions of the brain (Gaetz, 2004; Fritz et al., 2005). To prevent pain and distress and rapidly cause death, the methods should target the cerebral cortex, responsible for consciousness, and the brainstem, responsible for aspects of consciousness and respiratory and cardiac function (Shaw, 2002; Gaetz, 2004). The absence of brainstem reflexes has been used to determine insen-

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sibility in pigs (Anil, 1991; Vogel et al., 2011) and to confirm brain death in humans when absence of reflexes is combined with apnea (Wijdicks, 2001).

Recent work evaluating a nonpenetrating captive bolt (NPCB), Zephyr-Rabbit Stunner (**Zephyr-RS**), found that the method rendered piglets immediately insensible; however, some piglets showed signs of returning to sensibility (Widowski et al., 2008). Subsequently, the bolt head of the Zephyr-RS was modified to a conical shape and depth of depression was increased. This newly modified gun, Zephyr-Euthanasia (**Zephyr-E**), was designed specifically for euthanasia purposes, whereas the Zephyr-RS model was intended to stun rabbits before exsanguination during slaughter.

The objective of this study was to determine the effectiveness of the Zephyr-E for euthanasia of neonatal piglets. Latencies to insensibility and cardiac arrest were outcomes used to determine whether the technique was effective and humane. Postmortem assessments were completed to determine the degree of traumatic brain injury (**TBI**) caused by the Zephyr-E.

METHODS

All procedures were approved by the Animal Care Committee at the University of Guelph. A total of 100 low viability, neonatal piglets ($1.04 \text{ kg} \pm 0.03 \text{ SE}$) less than 72 h of age were used in the study. All piglets 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.

Euthanasia Device and Procedures

The Zephyr-E (Fig. 1) is a pneumatic nail gun [NS 100A 1/4-inch (6.35 mm) narrow crown stapler, Porter Cable Corporation, Jackson, TN] that is modified to hold a conical nylon bolt head (diameter: 2.5 cm, length: 3.8 cm) attached to a cylindrical bolt (diameter: 0.8 cm; Erasmus et al., 2010a). The nylon bolt head recesses 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 to 120 pounds per square inch (**psi**)]. 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.

Data were collected on 1 university research farm and 3 farrowing units from a commercial farm in Iowa. Ten stock people who were routinely responsible for performing euthanasia at the farms were trained to use the Zephyr-E. Stock People 1 and 2 were from the research



Figure 1. Photo of the Zephyr-Euthanasia 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.

station, Stock People 3 to 5 were from Farrowing Unit 1, Stock People 6 and 7 were from Farrowing Unit 2, and Stock People 8 to 10 from Farrowing Unit 3. Each stock person manually restrained each piglet on its sternum on a hard, flat surface (counter top). Two shots were administered in rapid succession on the frontal bone (AASV and NPB, 2009), followed immediately by 1 shot delivered to the back of the skull behind either ear. All three shots were typically completed in less than 4 s.

Ante Mortem Data Collection

Immediately after Zephyr-E application, piglets were assessed by the researcher for signs of sensibility using brainstem reflexes: corneal reflex, pupillary light reflex, jaw tone, and response to nose prick. The corneal reflex was tested by touching the surface of the eye and monitoring for a blink response. The pupillary light reflex was tested by shining a light into the eye of the piglet and observing for a dilated pupil that did not constrict in response to the light stimulus. Jaw tone was tested by gently pushing on the lower jaw muscle to examine whether there was any resistance to the downward motion. A needle prick to the nose was used to evaluate a withdrawal response to a painful stimulus.

Onset and duration of clonic and tonic neuromuscular leg spasms (convulsions) and the presence of respiration were monitored by visual assessment. The duration of clonic convulsions (**CC**) began at the onset of leg paddling and ended when 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 piglet became completely limp and motionless. Presence and duration of heartbeat (**HB**) were determined by palpa-

tion or auscultation. Cardiac arrest was determined to have occurred when no discernible heartbeat 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 piglets exhibiting signs of returning to sensibility, the Zephyr-E was immediately reapplied. If the method did not successfully induce cardiac arrest within 15 min, an alternative euthanasia technique was to be used. Anesthetic overdose [pentobarbital sodium (340 mg/mL) 0.3 mL/kg IC, Euthanasol, Schering-Plough (now Merck Animal Health) 16750 Route TransCanadienne, Kirkland, QC] was used as a secondary method on the research farm, and exsanguination was used on the commercial farm.

Postmortem Data Collection

A subset of the piglets that were euthanized at the research farm ($n = 10$) were scanned by computed tomography (CT) within 3 h of euthanasia by the Department of Clinical Studies, Ontario Veterinary College. Scans were made from the tip of the nose to the third cervical vertebrae using a GE LightSpeed 4 slice scanner (General Electric Company, Mississauga, ON). Images were acquired as a helical study in soft tissue and bone algorithms with a 1.25-mm slice thickness and a 0.75-mm interval with a small field view at 120 kVp and 100mAs. Images were later evaluated by a veterinary radiologist (S. G. Nykamp) and scored for hemorrhage severity (0 = no hemorrhage, 1 = mild, 2 = moderate, 3 = severe) and fracture displacement (FD). Fracture displacement was recorded as the distance (millimeters) between the normal position of the cortical bone to where the cortical bone had been displaced. Skull thickness (millimeters) was also measured.

Before dissection, each piglet ($n = 100$) was weighed (BW in kilograms) and crown to rump length was recorded (centimeters) to calculate body mass index [BMI = mass (kilograms)/length (meter²)]. Piglets were then scored macroscopically during gross dissection for brain hemorrhage and skull fracture (SK). Macroscopic scoring was based on a 0 to 5 point scale (Table 1), adapted from Erasmus et al. (2010b). Skull fracture was assessed by removing the scalp and examining the entire dorsal surface of the skull. Subcutaneous (SC), subdural dorsal (SDD), and subdural ventral (SDV) scores were used to assess severity of hemorrhage. Hemorrhage underneath the scalp on the dorsal surface of the skull after the scalp was removed from the medial canthus of the eyes to the base of the skull was scored and classified as SC. Hemor-

Table 1. Macroscopic scoring system¹

Score	Fracture score ² description	Hemorrhage score ³ description		
	SK	SC	SDD	SDV
0	No fractures, intact skull	No hemorrhage		
1	hairline fractures, no separation of bone	<25% of surface area covered		
2	one to two complete fully separated fractures or single depressed fracture	26 to 50% of surface area covered		
3	more than just a single depressed fracture, 3 to 5 complete fractures	51 to 75% coverage		
4	>5 complete fractures, fully fragmented skull	76 to 99% coverage		
5	N/A	complete coverage		

¹Adapted from Erasmus et al. (2010b).

²Fracture score: skull fracture (SK).

³Hemorrhage scores: subcutaneous (SC), subdural-dorsal (SDD), subdural-ventral (SDV).

rhage on the entire dorsal surface of the brain after skull and dura were removed was scored and classified as SDD, and SDV was assessed by scoring the hemorrhage on the entire ventral surface of the brain once the brain was lifted from the skull and the dura was removed.

After gross macroscopic evaluations, brains of the 10 piglets that underwent CT scans were removed and placed in 10% buffered formalin for at least 7 d. Once fixed, the brains were divided down the midline into 3 coronal sections, one section from each of the following regions of the right hemisphere: cerebral cortex, midsection of the brain including the thalamus, and brainstem. Each tissue sample was embedded in paraffin, sectioned, and stained with haematoxylin and eosin using standard techniques (Animal Health Laboratory, University of Guelph, Guelph, ON). The 3 brain sections were microscopically examined and scored without knowledge of treatment or location by a veterinary pathologist (PV Turner) to determine the degree and location of subdural (SDH) and parenchymal (PH) brain hemorrhage. 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). Differences in damage among the 3 brain regions were assessed by comparing scores from each region. The degree of overall microscopic damage for both SDH and PH was assigned a value by using the highest score for SDH and PH in any of the 3 regions from an individual piglet.

Statistical Analyses

All statistical analyses were computed in SAS 9.2 (SAS Inst. Inc., Cary, NC). Mixed model analyses of variance were used to test for overall differences among stock people for mean durations of CC, TC, and HB with stock person as a fixed effect nested within farm. To account for piglet differences, BW was included in the model as a covariate and farm as a random effect.

Table 2. Microscopic scoring system¹

Score	Percentage slide coverage ²
0	None (0%)
1	Minimal (<5%)
2	Mild (<10%)
3	Moderate (<30%)
4	Marked (>30%)

¹Adapted from Erasmus et al. (2010b).

²Subdural and parenchymal hemorrhage were determined by the percentage of the total slide covered by hemorrhage.

Durations of CC, TC, and HB were dependent variables. Baxter et al. (2008) reported BMI was significantly correlated with stillborns as well as postnatal piglet mortality. In an attempt to account for growth variation and morbidity effects, the mixed model of stock person nested within farm was repeated using BMI as a covariate in place of BW. The durations of CC, TC, and HB were log transformed to normalize the data. Log transformed means and standard errors for stock person mean duration of HB are presented in the results.

A linear regression analysis was used to test for linear relationships FD and BW. This regression analysis was repeated with FD and BMI. Microscopic hemorrhage data was rank transformed (Akritas, 1990) using proc RANK, and one-way ANOVA was run on the ranked data to test the effects of brain sections on hemorrhage scores. Statistical significance was defined as $P < 0.05$ for all analyses.

RESULTS

Ante Mortem Sensibility Assessment

All 100 piglets were rendered immediately insensible without any piglets showing signs of return to sensibility, and death was achieved without a secondary step in 94 of 100 piglets. Although the remaining 6 piglets showed no signs of sensibility, cardiac arrest was not achieved within the 15-min observation period. Four of the piglets required a secondary step (exsanguination) due to the presence of a faint, irregular heartbeat at the 15-min endpoint, and the other 2 piglets required anesthetic overdose as an alternative euthanasia method due to sustained, sporadic convulsions.

The average age, weight, and BMI of the piglets were 2.1 d \pm 0.08 SE, 1.04 kg \pm 0.03 SE, and 11.6 \pm 0.2 SE, respectively. The average duration of CC was 101 s (\pm 7.4 SE). The average duration of TC was 229 s (\pm 9.18 SE). The average HB duration was 420 s (\pm 13.57 SE). Durations of CC, TC, and HB were classified into 1-min intervals and plotted across time to show the cumulative percentage of piglets ceasing CC, TC, or HB at any one time point (Fig. 2). Body mass index had a

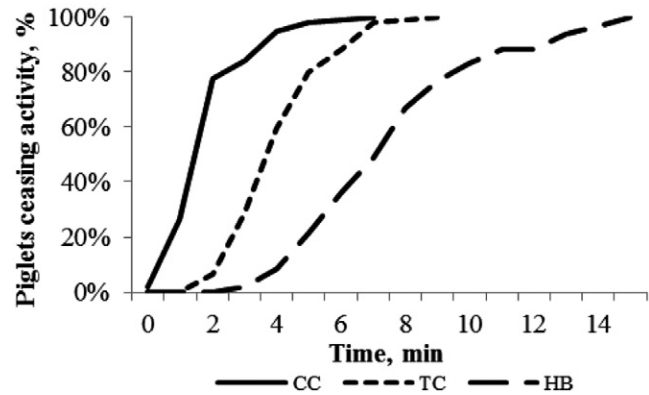


Figure 2. Cumulative percentage of piglets ceasing clonic convulsions (CC), total convulsions (TC), and heartbeat (HB) across time. Time point 0 indicates the time immediately following Zephyr-Euthanasia application. The 6 piglets that required a secondary step and were not included in the duration results ($n = 94$). Ninety-five percent of piglets ceased CC in <4 min, with all piglets ceasing CC in <7 min. Ninety-eight percent of piglets achieved brain death in <7 min, with all remaining piglets ceasing TC in <9 min. Eighty-eight percent of piglets achieved cardiac arrest in <11 min.

significant negative effect on the duration of CC ($P = 0.023$; $R^2 = 0.03149$); however, based on the R^2 value, BMI accounted for a small degree of variation. As BMI increased, the duration of CC decreased.

There were significant effects of stock person on the duration of HB when BW was a covariate ($P = 0.0053$) and when BMI was a covariate ($P = 0.0059$). Figure 3 illustrates the least square means (\pm SEM) for duration of HB of the 10 piglets euthanized by each stock person. There were no differences across stock people for durations of CC or TC.

Postmortem Damage Assessment

Based on CT scan results, the mean hemorrhage severity score was 2.3 (\pm 0.3 SE), with moderate to severe hemorrhage noted in 9 of 10 piglets. One piglet had no evidence of hemorrhage even though severe SK was present. Of the remaining 9 piglets, 6 had only extradural hemorrhage, whereas 3 had both extradural and PH present. Mean FD was 6.2 mm (\pm 0.7 SE). There was no significant linear relationship between FD and BW ($P = 0.8408$) or BMI ($P = 0.6439$). Mean skull thickness was 2.3 mm (\pm 0.2 SE).

The frequencies of macroscopic scores are presented in Table 3. All piglets exhibited moderate to severe SK and some degree of hemorrhage; 97% of piglets had moderate to severe hemorrhage. A score of 4 was the most frequent score for every hemorrhage category.

Histological analyses indicated SDH and PH were present in at least 1 section from every piglet brain scored ($n = 10$; 3 coronal sections each). When comparing the 3 sections from each brain, there were no significant differences for SDH ($P = 0.2302$); however, the cerebral

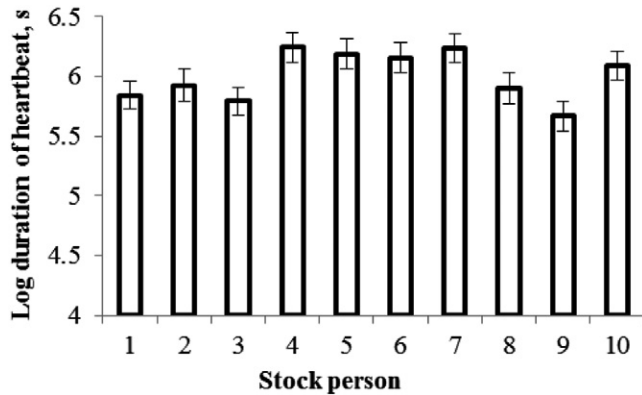


Figure 3. Least square means (\pm SEM) for the duration of heartbeat (HB) of the 10 piglets euthanized by each stock person. Stock person had a significant effect on HB ($P = 0.0053$). The majority of stock people had differing means from at least one other person, with Stock People 3 and 9 averaging the shortest durations and differing from 4 or more other stock people. Raw mean duration of heartbeat is 420 s (\pm 13.6 SE).

cortex had more severe scores for PH ($P = 0.0328$) compared to the midbrain and brainstem sections (Table 4). All brains sections had SDH present, whereas PH was found in all 10 brain sections from the cerebral cortex, but only in 6 sections from the midbrain and 7 sections from the brainstem.

The highest SDH and PH scores from each piglet brain, regardless of the section of origin, indicated moderate to severe SDH in 100% of piglets, and moderate to severe PH in 60% of piglets.

DISCUSSION

The Zephyr-E was highly effective for providing a humane death for the piglets euthanized in this study. All 100 piglets were rendered immediately insensible, without any showing signs of return to sensibility. This was an improvement from the rabbit stunner evaluated by Widowski et al. (2008), in which 13% of piglets showed signs of return to sensibility. This difference between devices emphasizes the importance of the design of the bolt head and bolt length. The pneumatic device used in the previous study was also designed to deliver 120 psi, but had a rounded bolt head and the bolt length was less than half the length of the bolt used in the Zephyr-E.

Time to insensibility and time to brain death are considered to be some of the most important criteria for assessing euthanasia methods because they ensure that the animal is no longer experiencing any pain or distress and is unable to regain function (American Veterinary Medical Association, 2007). For humans, brain death is diagnosed mainly through evidence of loss of brainstem function (Wijdicks, 1995). Clinical criteria include loss of consciousness, loss of motor responses to pain, loss of brainstem reflexes, and apnea. Confirmatory testing can be done by electroencephalography (EEG) and is indi-

Table 3. Distribution of macroscopic hemorrhage scores

Score ¹	0	1	2	3	4	5
SC	0	3	20	22	35	20
SDD	0	1	22	34	35	8
SDV	0	0	17	32	42	6
SK	0	0	67	33	0	N/A

¹Scores: subcutaneous (SC), subdural-dorsal (SDD), and subdural-ventral (SDV) hemorrhage and skull fracture (SK). $N = 100$ for SC, SDD; SK $n = 100$; and $n = 97$ for SDV.

cated by an isoelectric recording or loss of somatosensory or audiovisual evoked potentials (Wijdicks, 2001). In animal studies, the *gold standard* for indicating brain death is often the presence of an isoelectric recording during EEG collection; however, this technique is impossible when applying physical methods for euthanasia due to the potential for damage to the electrodes and the electrical interference caused by convulsions (Erasmus et al., 2010c). In the present study, brain death was defined by the end of TC, at which point all movement had stopped, and all breathing and reflexes were absent since the time of Zephyr-E application. This occurred within 5 min in 80% of piglets and <9 min in all 100 piglets. The cessation of all movement along with the sustained absence of breathing has been observed to occur at the same time as cessation of electrical activity in the brain in poultry (Dawson et al., 2007, 2009; McKeegan et al., 2011; Turner et al., 2012). Although not specifically recorded, it was anecdotally noted that, while the pupil remained nonresponsive to light throughout the observation period, it became fully dilated at or before the cessation of TC in the piglets in the current study. In humans, a nonresponsive, midsized to fully dilated pupil, coupled with apnea, is considered to be indicative of brain death (Hills, 2010).

Cardiac arrest is often used to determine absolute time of death; however, even after irreversible brain death, blood pressure frequently fluctuates and the heart can continue to beat in a reduced manner for a period of time (Conci et al., 2001). Turner et al. (2012) found hearts continued to beat for several minutes following

Table 4. Distribution of microscopic hemorrhage scores within each brain section

Brain section ¹	Cerebral cortex				Midbrain				Brainstem						
	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
SDH	0	0	2	6	2	0	0	1	8	1	0	3	2	4	1
Section Means (\pm SE)	3.0 \pm 0.21				3.0 \pm 0.12				2.3 \pm 0.33						
PH	0	5	0	4	1	4	3	3	0	0	3	4	2	1	0
Section Means (\pm SE)	2.1 \pm 0.38 ^a				0.9 \pm 0.28 ^b				1.1 \pm 0.31 ^b						

^{a-b}Within a row, means without a common superscript differ ($P < 0.05$).

¹Each brain was divided into 3 coronal sections. $n = 10$ brains, 30 sections for both SDH and PH.

²Scores: subdural (SDH) and parenchymal (PH) hemorrhage.

brain death as confirmed by an electrocardiogram and EEG when poultry were euthanized using carbon dioxide (CO₂). In our study, the piglets were monitored until the last heartbeat was determined, even if the pattern was faint or irregular. With this conservative approach, the Zephyr-E caused cardiac arrest on average in 7 min, which is similar to the 6-min time frame with CO₂ euthanasia (Chevillon et al., 2004), and within the range reported for other NPCB guns causing death within 2 to 7 min (Widowski et al., 2008; J. A. Woods, Iowa State University, Ames, unpublished data). Time to cardiac arrest was reported to be less than 3 min (Widowski et al., 2008) and less than 10 min (Chevillon et al., 2004) following blunt force trauma (**BFT**). Unlike the NPCB application used by Finnie et al. (2003), which failed to cause cardiac arrest in all pigs, the Zephyr-E successfully achieved death in a single step in 94% of piglets. This difference among studies emphasizes the importance of placement, as the temporal position was used by Finnie et al. (2003), which is not anatomically appropriate for swine due to the position of the brain. Woods et al. (2011) reported success as a single step euthanasia technique with a cartridge-based NPCB (the Cash Euthanizer) when applied to the forehead for suckling and nursery age swine. Although 4% of piglets in the current study required a secondary euthanasia step to reach full cardiac arrest, heartbeat at the 15-min endpoint was irregular and faint. These piglets did not show any signs of returning to sensibility, had ceased movement, and hence, would have likely progressed to full cardiac arrest without a secondary step if a 15-min endpoint was not in place.

Regarding the visual aesthetics of convulsions, the clonic paddling stage is most visible and arguably the most disturbing to an unfamiliar audience. In our study, these paddling movements ended within 2 min for 80% of piglets and within 7 min for all 100 piglets observed. It is important to emphasize that these grand mal convulsive movements are involuntary neuromuscular responses indicative of epileptiform brain activity that occurs following severe concussion (Shaw, 2002) and during electrical stunning in pigs (McKinstry and Anil, 2004). To date, varying degrees of these convulsive movements have been reported following application of all available euthanasia techniques for piglets of this size including BFT and NPCB (Chevillon et al., 2004; Widowski et al., 2008; J. A. Woods, unpublished data) and CO₂ (Raj and Gregory, 1996; Sutherland, 2010; Sadler, 2013). The convulsive period was slightly longer than the 1 to 1.5 min typically seen with BFT (Widowski et al., 2008; Chevillon et al., 2004), but was similar to other NPCB devices falling within the range or 1 to 4 min reported by J. A. Woods (unpublished data) and similar to the 2 min reported by Widowski et al. (2008). Two of

the 100 piglets in the current study were administered a secondary euthanasia step due to sustained involuntary movements. Although both piglets were determined to be brain dead, abnormal convulsion patterns persisted even after additional shots were fired. One piglet continued spontaneous movements even after anesthetic overdose. In humans, it is reported that spontaneous body movements, such as head turning, flexion at the waist, and arm raising may occur when patients are deemed to be brain dead, and that handling the patient may initiate movements that are generated by spinal reflexes (Wijdicks, 2001). Humans and other animals are also 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).

Verbal feedback from stock people on each of the farms mentioned a common experience that moribund or disproportionate (long and skinny with a large head) piglets typically took “longer to die” during routine euthanasia on the farm. These reports are supported by the significant inverse relationship between BMI and duration of CC found in our study and may be related to a difference in neurologic or physiologic development of piglets exhibiting a low BMI, as suggested by Baxter et al. (2008).

Severe SK and brain hemorrhages were consistently observed in our study. The presence of contusions directly below the site of SK, as well as intracranial hematoma, which is commonly seen in fatal traumatic brain injuries in humans (Young and Destian, 2002), were confirmed by the CT scans. Although brain concussions can occur without SK (Shaw, 2002), SK with similar severity have been reported in association with fatal TBI in humans and rats (Tseng et al., 2011; Viano et al., 2012). Skull fragments embedded in the brain can also directly damage brain tissue, altering regional cerebral blood flow (Nedd et al., 1993). The severe SK and widespread brain hemorrhage, easily visible to any observer during gross dissection, was similar in magnitude to the lethal damage reported by Widowski et al. (2008) and Whiting et al. (2011). In addition to the subcutaneous and subdural hematomas evident in all piglets in our study, widespread hemorrhage was also visible on the opposite side of the brain (ventral brainstem). This type of coup–contrecoup contusion (Gaetz, 2004) suggests that the Zephyr-E induced sufficient concussive force to physically jolt the brain within the cranium. It is unlikely to have occurred secondary to blood pooling, since blood clots were removed from the surface before scoring to reduce inflated scores. In contrast to the very mild, localized PH reported in piglets surviving head impact (Duhaime et al., 2000; Finnie et al., 2003), the subdural and parenchymal microscopic damage seen in the present

study was widespread and categorized predominantly as moderate, confirming the presence of significant damage to the brainstem as well as damage to the cortex and subcortical tissue. Evidence of subdural and parenchymal rupture of blood vessels and surrounding cell death in all of the piglets examined suggests the combination of a focal and diffuse injury, which is commonly seen in moderate to severe TBI (Gaetz, 2004). Overall, all three modes of postmortem assessment confirm moderate to severe widespread damage was caused by the Zephyr-E.

There are few reports in the literature on the repeatability of euthanasia techniques performed by multiple stock people. Unlike Widowski et al. (2008), who reported no significant differences in cessation of leg movements or heartbeat among stock persons using a NPCB on piglets, the current study found variation in the duration of heartbeat when euthanasia was performed by different individuals. Despite this, the degree of variation in HB duration was minimal and did not reduce the effectiveness of the technique, as all piglets died in a timely manner.

Feedback from stock people was informally acquired through a brief survey. All participating stock people were experienced and comfortable with traditional BFT for neonatal piglets, but were especially interested to test the Zephyr-E on larger, weaned piglets. This coincides with the understanding that a gap exists in available methods, in particular for the nursery stage of production. Overall, stock people rated the Zephyr-E highly as a euthanasia technique, with an average rating of 8.7 (± 1.6 SE) on a 10-point scale, where 1 was rated as poor and 10 was rated as excellent. Stock people reported that the Zephyr-E was very easy to use, provided a high degree of confidence that the technique was successful, and was less esthetically objectionable than BFT, which was the technique most commonly used on the farms involved in this study. In terms of the aesthetics of the technique, from casual observations it was noted that a noticeable bruise was present on the forehead of most animals with very few incidences of skin puncture, estimated at <10%, and occasional external bleeding from an ear or the nostril in approximately 30% of piglets.

In conclusion, based on evidence to support immediate insensibility, a timely death, severe TBI, and positive stock person feedback, the Zephyr-E is a practical, humane alternative for on-farm euthanasia of neonatal piglets. These results confirm recommendations provided by the National Pork Board, AASV (AASV and NBP, 2009), and OIE (2011) that a NPCB may be used as a single step euthanasia technique for neonatal piglets up to 5 kg.

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