© 2017 Universities Federation for Animal Welfare The Old School, Brewhouse Hill, Wheathampstead, Hertfordshire AL4 8AN, UK www.ufaw.org.uk

Humane euthanasia of neonates I: validation of the effectiveness of the Zephyr EXL non-penetrating captive-bolt euthanasia system on neonate piglets up to 10.9 kg live-weight

A Grist*, JC Murrell, JL McKinstry, TG Knowles and SB Wotton

School of Veterinary Sciences, University of Bristol, Langford House, Langford, North Somerset BS40 5DU, UK * Contact for correspondence and requests for reprints: Andy.Grist@bristol.ac.uk

Abstract

To determine if mechanical blunt force trauma using a non-penetrating captive bolt was a viable method of producing an immediate stun/kill in neonate piglets (Sus scrofa domesticus) as an alternative to manual blunt force trauma. Piglets (n = 60) were acquired from a local producer and allocated to one of five weight ranges: birth weight to 3 kg (n = 12); 3 to 5 kg (n = 11); 5 to 7 kg (n = 13); 7 to 9 kg (n = 13); and 9 to 11 kg (n = 11). These piglets with an average live-weight of 6.1 kg were anaesthetised and electroencephalogram (EEG) recording electrodes inserted sub-dermally over the right cranium to allow recording of Visual Evoked Potentials (VEPs). Following recording of baseline VEPs in the anaesthetised state, the piglet was shot once in the frontal-parietal position with a Bock Industries Zephyr EXL non-penetrating captive bolt powered by 120 psi air pressure. Movement scoring, behavioural indices of loss of brain function and VEPs were monitored throughout. VEPs were lost immediately in all piglets shot when the head was resting on a hard surface. This experiment demonstrates that mechanical blunt-force trauma, using a single-shot, non-penetrating captive bolt, such as the Zephyr EXL, provides for an immediate stun kill in neonate piglets up to 10.9 kg live-weight. This immediacy of action, combined with reproducible effects will improve the welfare of piglets to be subjected to on-farm euthanasia due to disease, ill-thrift or other commercial concerns.

Keywords: animal welfare, captive bolt, euthanasia, mechanical stunning, piglet, Visual Evoked Potentials

Introduction

Modern pig (Sus scrofa domesticus) production has an inherent requirement for the humane euthanasia of neonate piglets for various reasons, including herd productivity, disease and under performance. In the United Kingdom, preweaning mortality averages 14.18% (Agriculture and Horticulture Development Board Report 2015) indicating that one in twelve piglets in a litter may require dispatch before the average weaning age of 26 days (average piglet weight 7 kg). The traditional method of dispatch is manual blunt-force trauma (MBFT), either through holding the piglet by the hind legs and hitting the head against a hard object or using some form of blunt-force trauma such as a 'priest' (a heavy-ended baton also known as a gamekeeper's or poacher's priest) or a hammer. Manual blunt-force trauma as a humane method of euthanasia has several issues; firstly, it relies upon the ability of the operator to successfully perform the action, secondly the effects may not be reproducible and, thirdly, stockmen do not like performing the operation unless the animal appears ill and the method of euthanasia was perceived as being less painful to the animal (Matthis 2004; Mort et al 2008; Whiting & Marion 2011; Whiting et al 2011). Mechanical killing via blunt-force

trauma using a non-penetrating captive-bolt device has the advantage of reproducibility, less reliance upon operator ability and with training, including the identification of post mortem movement that indicates an effective stun/kill, enhanced operator acceptability.

Non-penetrative mechanical stunning relies upon imparting kinetic energy to the cranium to produce concussive effects within the brain, based on the velocity of the impact rather than the mass of the object (Daly et al 1987). The concussion produced by this impact is often associated with both haemorrhaging at the impact site ('coup') and further haemorrhaging opposite the impact site ('contra-coup') (Ommaya et al 1971). This is due to the rotational and differential acceleration of the brain within the cranium (Ommaya & Gennarelli 1974). Shearing forces are produced within the brain by the pressure waves producing vacuolation (Finnie 1995; Finnie et al 2003), disruption of synaptic transmission (Gregory 1998) and depolarisation of neurons away from the impact site (Somjen 2001). Shaw (2002) also discusses the effects of sudden change in intra-cranial volume, brain compression and pressure waves following compression of the skull, with the pressure waves terminating at the brainstem and cranio-cervical junction. The most common

https://doi.org/10.7120/09627286.26.1.111 Published online by Cambridge University Press



cause of death following brain injury is subdural haemorrhage due to direct injury to the cortical arteries and veins by the object, contusion and pulping of the cerebrum, or tearing veins that bridge the subdural space between the brain surface and the dural sinuses (Millman 2010).

There were initial concerns that the incomplete sutures in the newborn piglet that provide for cranial deformation during parturition may provide a form of elastic protection from the effect of Blunt Force Trauma (BFT), in effect absorbing the blow. Previous studies (Defra MH0116, S Wotton, personal communication 2016) found that with a non-penetrating captive bolt (NPCB), the skull development of the neonate piglet is sufficient for the transfer of kinetic energy to the brain to produce a stunkill. Research by Armstead (1999) also demonstrated that newborn piglets were particularly sensitive to brain injury.

A study to assess the effectiveness of an NPCB for euthanasia of suckling and weaned piglets using the Bock Industries Zephyr E pneumatic, non-penetrating, captive bolt was undertaken by the University of Guelph. This study used visual signs of sensibility and behavioural indicators including loss of rhythmic breathing, corneal reflex and response to painful stimuli to assess the effectiveness of the stun combined with post mortem examination of the head to assess skull and brain damage following a two or three shot technique (Casey-Trott *et al* 2013, 2014). Following this research, Bock Industries upgraded the Zephyr E to the Zephyr EXL which has a higher velocity, when operated at 120 psi, than the Zephyr E and hence develops a higher kinetic energy (27.7 cf 20 J) (J Lines, personal communication 2015) to allow a single-shot technique to be applied.

This current study sought to evaluate the effectiveness of the Zephyr EXL on neonatal piglets (n = 60) using the loss of Visual Evoked Potentials (VEPs) as an indicator of cortical brain death (Gregory & Wotton 1984: Guerit 1999) followed by measurements of post-stun movement, post mortem examination of fracture patterns and macroscopic examination of the brain.

Materials and methods

Zephyr EXL velocity

The Zephyr EXL velocity was measured by two methods to provide evidence of the velocity and hence kinetic energy (KE = $\frac{1}{2}$ mv²; where m = mass of projectile and v = velocity) produced by the non-penetrating captive bolt to give a guideline figure for any future recommendations. One velocity measurement was assessed by the manufacturer (Bock Industries, PA, USA); the Zephyr-EXL was hoseconnected (20 ft) to a 120-psi air pressure supply and mechanically fastened to a granite table with the bolt firing in the horizontal position. Using high speed (10 kHz) analogue videography (Fastcam SA1.1, Proton, San Diego, CA, USA) combined with custom digitising frame analysis software (Matlab, Mathworks, Torrance CA, USA) to directly calculate Zephyr-EXL bolt velocity as a function of bolt displacement. Based on the mean (± SEM) of three trials, the maximum velocity was 27.4 (\pm 0.1) m s⁻¹ (26 J).

These figures were confirmed by bench-testing the device at 6 to 8.1 bar (87 to 117 psi) and a prediction of its performance

at 120 psi was made. Before firing, the apex of the percussive head cone is retracted 29 mm from the contact position, within the barrel. The bolt is free to travel to a point 30 mm beyond the contact position without any reciprocating buffers, ie free-flight.

The moving components of the Zephyr EXL in normal use comprise a steel bolt and a plastic hammer head weighing a total of 62 g. The device was tested with combined bolt, projectile holder and projectile masses of 69 and 99 g. Maximum bolt velocity during the stroke was measured. The bolt energies at the higher bolt mass differed from the lower mass by less than 4% despite the 43% change in mass. Therefore, the energies of the bolt at the lower test mass (69 g) were taken to be the same as the energy in use (mass 62 g).

Bolt energies were found to be 20.6 J at 6 bar (87 psi) and 27.2 J at 8.1 bar (117 psi) indicating that the energy at 120 psi would be expected to be 27.7 J (J Lines, personal communication 2015).

Study animals

All procedures were carried out in the University of Bristol, School of Veterinary Sciences in the United Kingdom (UK) under the provisions of the Animals (Scientific Procedures) Act 1986 and with the approval of the University of Bristol's Ethical Review Process.

Healthy piglets were purchased from a local farm (n = 62)and assigned to one of five weight ranges: birth weight to 3 kg; 3 to 5 kg; 5 to 7 kg; 7 to 9 kg; and 9 to 11 kg; the average live-weight of the piglets in this study was 6.1 kg. Two of the piglets (numbers 5 and 6) were not used as they were found to be over the upper weight range of the trial. These piglets were anaesthetised with sevoflurane (SevoFlo, Abbott Animal Health, UK) vaporised in oxygen delivered via an Ayre's T-piece breathing circuit. In order to induce anaesthesia, piglets were gently restrained and an appropriately sized close-fitting face-mask (Ace Veterinary Supplies Ltd, UK) was attached to the breathing circuit and placed over the muzzle. Sevoflurane was delivered to the face-mask at a dialled vaporiser setting of 8%, using an oxygen flow rate of 3 L min⁻¹. Following induction of anaesthesia, signalled by loss of voluntary movement, recumbency and loss of the palpebral reflex the concentration of sevoflurane was reduced to 2.5%. Anaesthesia was maintained with this concentration of sevoflurane using the face-mask and breathing circuit for the duration of the procedure. The electrocardiogram was recorded immediately after induction of anaesthesia using three surface ECG electrodes attached to the right and left forefeet and left hind foot of the piglet and a multi-parameter monitor (Datascope Passport 2, Mindray, DS USA).

Three 13-mm disposable sub-dermal needles (SD51, Unimed Electrode Supplies, UK) were inserted subcutaneously into the head of the animal in accordance with the techniques described for sheep and pigs (Gregory & Wotton 1983; Wotton & Gregory 1986). The negative electrode was placed 0.8 to 1.4 cm rostral to the lamboid suture; the earth electrode 1.5 cm rostral to the negative electrode and the positive 1.5 cm rostral to the earth electrode, level with the rear canthus of the eye (Wotton & Gregory 1986). Following epidural implantation, the left eye of the animal was taped open to expose the pupil. (Figure 1).

^{© 2017} Universities Federation for Animal Welfare

Visual Evoked Potential recording

The EEG electrodes were connected to a DAM 50 differential amplifier (World Precision instruments, FL, USA) before recording at a sampling frequency of 1,024 Hz using Powerlab 4/35 (ADinstruments, UK). The band pass filter was between 0.1 and 100 Hz and the gain was set at 100. The scope software was set up to run initially without the light shining on the pig and no responses were seen allowing the conclusion that the VEPs that were recorded were responses to the visual stimulus rather than artificial synchronised responses. VEPs were recorded with potentials being triggered and powered by an SLE Photic Stimulator at two flashes per second under normal lighting conditions. All epochs were recorded and stored using Scope4 software (ADInstruments, UK) which records and analyses signals that are time-locked to the photic stimulus, and allows averaging of repetitive signals time-locked to a stimulus to give an overall average waveform from a number of stimulus repetitions. Each waveform post-stun was an average of 16 epochs of 200 ms duration recorded at a stimulus rate of two flashes per second, ie over an 8-s period. The following VEP was analysed over the subsequent 16 epochs. This process continued for 64 epochs post-stun to ensure that VERs were lost. The pre-stun averages were reviewed immediately to verify that there was a repeatable VEP in all animals (similar to that shown in Figure 2).

At the end of EEG data collection, following confirmation of brain death, administration of sevoflurane and oxygen was stopped.

Once the pre-recordings were completed, the animals' heads were held manually against the operating table and shot once using the Bock Industries Zephyr EXL pneumatic captive-bolt gun in the parietal position by a senior researcher. The gun was fired using compressed air at 120 psi delivered by a Scheppach HC51 oil-lubricated 50 L air compressor (Scheppach, DE, USA).

VEPs were recorded continuously for 3 min post-shot to ensure they did not return, ie to verify the death of the animal. Following stunning, subjective evaluations were made on the effectiveness of the stun, ie loss of brainstem reflexes, such as rhythmic breathing, loss of corneal reflex (no response to corneal stimulus) and palpebral reflex (no response to stimulation of the eyelid). These observations were made continuously throughout the 3-min recording period looking for any return of rhythmic breathing or agonal (spinal-induced) gasping. The level of post-stun movement (enhanced spinal reflex activity) was subjectively assessed and recorded on a scale of 0 to 3, based on the descriptors in Table 1.

Once completed, the VEP epochs were stored and analysed at a later date using Scope4 software. Sequences of 16 responses to photic stimulation were averaged together both pre- and post-stun. The post-stun averages were continued over a duration of 360 epochs (3 min) to identify the presence or absence of post-shot evoked potentials.

Figure I



Anaesthetised piglet with recording electrodes inserted for Visual Evoked Potential recording.

Post mortem analysis

Post mortem examination of the heads was carried out. After photographing the intact head using a Nikon D5100 digital camera (Nikon Corporation, Japan), the skin from the head was removed following a T-incision cranial to the shoulders and extending forward to the snout. The impact site was photographed before removal of any haematoma and the periosteum to expose fracture lines extending from the impact site. Photographs were taken of the fracture patterns to allow for later comparison. The heads were then hard frozen to facilitate sectioning on the medial plane for photography of cranial and brain lesions to be undertaken. The photographs of all the sagittal sections were assessed by two researchers without reference to age or weight group, with each sagittal section being scored for macroscopic damage to the brain with a scale adapted from Sharpe et al (2014): 0 = no damage; 1 = slight deformation; 2 = moderate deformation; and 3 = severe deformation of the area. The results were discussed by the two researchers and scores moderated. The areas examined for macroscopic damage were the frontal, parietal and occipital cerebrum including the structure of the lateral ventricle (Figure 3).

Statistical analysis

Below, we present simple summary statistics broken down by weight group. Correlations between variables were investigated using Spearman's Rho, a non-parametric test of correlation. A Jonckheere-Terpstra test, a non-parametric test, was used to investigate whether there was an ordered effect of median piglet weight upon movement score. All statistical analyses were carried out using IBM SPSS Statistics (v23).





Time (s)

An example of a pre-stun Visual Evoked Potential (VEP) (Piglet 54) illustrating a typical response following photic stimulation at time 0, indicating the visual pathway is functional.

Table I Subjective scoring system used to assess poststun/kill movement based on level of spinal reflex activity, ranging from 0 (no activity post-stun) to 3 (gross uncontrolled physical movement).

Score	Descriptor	Description
0	No activity	Very little movement
I	Mild activity	Some mild uncontrolled physical movement of limbs
2	Moderate activity	Considerable uncontrolled physical movement of the limbs
3	Severe	Gross uncontrolled physical movement

Results

Movement scores

The effect of piglet weight on movement score was analysed using a Jonkheere-Terpstra test for an ordered association with median weight within each movement score. The effect was significant with a standardised J-T statistic = -2.595; P = 0.009 (Figure 4). The analysis demonstrated that the median weight for movement score 0 was 10.3 kg, for score 1 the median piglet weight was 8.25 kg, score 2 the median piglet weight was 6.35 kg and for movement score 3 the median piglet weight was 5.5 kg.

Visual Evoked Potentials

Visual assessment of the VEP data showed that, of the 60 pigs shot, all were stunned and 59 (but see below) were killed by the blow, with immediate loss of VEPs post-stun as shown in Table 2. There were no further signs of VEPs in the first 32 s (64 epochs) post-stun (Figure 5). This abolition of VEPs remained throughout the 180-s recording period. All animals in groups birth to 3 kg (n = 12), 3 to 5 kg (n = 11), 5 to 7 kg (n = 13)

© 2017 Universities Federation for Animal Welfare

and 7 to 9 kg (n = 13) were killed immediately and showed no signs of recovery before death (Table 2), with the exception of the first piglet shot (piglet number 1, 9 to 11 kg group), which was stunned whilst the head was supported on a foam cushion (initially, to allow for differential acceleration of the brain within the cranium to produce severe concussion). Although demonstrating behavioural signs of being stunned, this animal showed VEPs throughout the 360-s recording period and required a secondary shot. Therefore, all subsequent animals were shot with their head manually supported against the solid surface of the operating table, after which all were successfully, immediately killed.

Recording was undertaken for a period of 50 ms before and 200 ms after delivery of the stimulus, and there was no evidence of waveforms from the previous stimulus overlapping the next stimulus, as evidenced by the flat baseline period in the 50 ms recording before the stimulus.

Post mortem analysis

Post mortem examination of the heads demonstrated a depressed fracture of the cranial plates corresponding to the impact footprint of the non-penetrating bolt, the depressed fracture being more defined in the heavier animals. In all weight ranges, the most common factor was a fracture extending caudally from the impact point bisecting the nuchal crest, interparietal and occipital bones and terminating at the atlanto-occipital joint.

On sagittal section within all groups there was evidence of haemorrhage throughout the cranial cavity with blood evident within the corpus callosum and surrounding the medulla oblongata and within the cerebellum. In all cases, the medial or frontal dorsal cerebrum showed damage after being crushed by bone plates, this can be seen by reference to differences with an unshot piglet head (Figures 6 and 7). Sagittal section of unshot piglet head (died on-farm) illustrating the areas examined for macroscopic damage. A) frontal cerebrum, B) parietal cerebrum, C) occipital cerebrum and D) lateral ventricle. These were scored on the basis of 0 = nodamage, I = slight deformation, 2 = moderate deformation and 3 = severe deformation of the area. Areas E-I (thalamus, midbrain, pons, medulla and cerebellum, respectively) were assessed for presence or absence of ecchymosis.







Movement score

The effect of individual piglet weight on post-shot movement score. Where 0 denotes no uncontrolled physical activity post-shot, 1 denotes mild uncontrolled physical activity post-shot, 2 denotes moderate levels of uncontrolled physical activity post-shot and 3 denotes severe uncontrolled physical activity post-shot. Illustrating the results of the Jonckheere-Terpstra test that showed that heavier piglets displayed less movement post-shot (P = 0.009).

116 Grist et al

Table 2 Results of the effect of the Zephyr EXL across piglet weight ranges on post-shot Visual Evoked Potentials (VEPs), breathing movements and spinal movement. One piglet from the 9 to 11 kg group was stunned but not killed, this was the only animal shot whilst its head was supported by a foam cushion, all others were subsequently placed against a hard surface.

Group	n	,	Weight (kg)		VEPs*			Breath	ing*	Move	ment sco	ore**
		Min	Max	Mean	Pre	Post	Return	Pre	Post	Agonal	Min	Max	Mean
0–3 kg	12	0.98	2.89	2.00	12	0	0	12	0	5	2	3	2.67
3–5 kg	П	2.37	4.90	4.31	П	0	0	11	0	0	2	3	2.91
5–7 kg	13	5.00	6.55	8.83	13	0	0	13	0	0	I	3	2.85
7–9 kg	13	7.50	8.90	8.28	13	0	0	13	0	I	0	3	2.23
9-11 kg	11	9.36	10.90	10.26	11	I	0	П	I	3	0	3	2.00
Total (%)	60			6.74	60	l (l.67)	0	60	I (I.67) 9 (15.00)			2.53
* 0 = abolished, 1 = present; ** see Table 1.													

Figure 5



Examples of Visual Evoked Potentials (VEPs) pre- and post-stun for piglet number 4 demonstrating loss of response to the photic stimulation (time = 0) post-shot. The figures in brackets are the number of stimuli post-shot (at two stimuli per second), for the VEPs shown. The lower plot shows the averaged VEP over the complete 180 s post-shot displayed against the pre-shot averaged signal. The event spike shows one of the stimulus flashes that were presented at a rate of two stimuli per second over 180 s after the shot.

© 2017 Universities Federation for Animal Welfare



Sagittal section of an unshot piglet head, demonstrating the frontal shot position and the major brain structures affected examined after the shot.

Figure 7

Group	Lesion example - External	Lesion example – Cross-section
0 to		
3 kg		
9 to		
11 kg		

General fracture pattern (skin and periosteum removed) and traumatic injury to brain in sagittal section of piglets shot with the Zephyr EXL.

Animal Welfare 2017, 26: 111-120 doi: 10.7120/09627286.26.1.111

118 Grist et al





Averaged cerebrum macroscopic lesion scores by treatment group, demonstrating a reduction in the subjective macroscopic lesions found in sagittal sections of heads as the piglets age. Four areas of the brain examined for macroscopic damage, the frontal, parietal and occipital cerebrum and the lateral ventricle. These were scored on the basis of: 0 = no damage, 1 = slight deformation, 2 = moderate deformation and 3 = severe deformation of the area.

Figure 8 illustrates the results of the blind study of the sagittal sectioned heads. The scores were tested for an overall correlation between individual piglet weight and the score for each of the four areas using a Spearman rank (r_s) correlation test. There was no significant correlation for frontal, parietal gave a significant negative correlation $(r_s = -0.274, df = 58; P = 0.034)$, occipital, showed no significant correlation $(r_s = -0.203, df = 52; P = 0.141)$ and lateral ventricle gave a significant negative correlation $(r_s = -0.390, df = 57; P = 0.002)$.

Discussion

General discussion

The use of the Zephyr EXL, as a euthanasia device for neonate piglets up to 10.9 kg, provides for immediate irreversible loss of Visual Evoked Potentials and hence cortical brain death (Guerit 1999) when the mechanical BFT is applied with the head resting on a hard surface. The macroscopic damage to the brain, both at the point of impact and contra-coup, suggests a level of unconsciousness and death due to the traumatic/concussive effect of the blow (Ommaya et al 1971). Duhaime et al (2000) found that in their model of focal brain injury the extent of brain damage following moderate scaled cortical impact increases with maturation and bodyweight, but did recognise that the findings may alter with severe mechanical trauma and the physiological stress imposed by other factors, such as subdural haematoma or diffuse inertial injury. Armstead and Kurth (1994) found a greater cerebrovascular physiological instability in younger animals following fluid-percussion injury.

Skull density and suture development does not appear to affect the efficacy of the impact of the Zephyr EXL, possibly due to the level of applied kinetic energy and the shot position being on the parietal bone. The depressed fracture pattern was more organised in the larger animals, due in part to the increasing bending stiffness of the parietal bone with age (Baumer 2010; Powell 2012). However, the efficacy of the BFT relies on the head being restrained on a hard surface. Piglet 1 was stunned, but not killed, indicated by the presence of VEPs following the shot. This was the only piglet shot with its head restrained on a foam cushion to allow for a degree of cranial acceleration, the remaining piglets being shot with their head manually supported against the hard surface of the operating table. This suggests that, with piglets, the use of a non-penetrating captive bolt to administer BFT relies less on differential acceleration of the brain within the cranium, but more a high velocity impact producing deformation of cranial bones and pressure waves throughout the brain corresponding to theories presented by Shaw (2002).

Movement post-shot

Movement post-shot is generated through enhanced spinal reflexes. The mechanical BFT so disrupts the function of the brain that the normal inhibitory influences, of the higher centres on the spinal cord, are lost before the spinal cord becomes exhausted and unresponsive. This loss of control by the higher centres over spinal reflexes results in an enhancement of their activity (Gregory 1993). Thus, post-shot convulsions can be produced at a spinal level and it may well be argued that they should be produced, as they are one of the indicators of brain dysfunction and hence an effective stun.

© 2017 Universities Federation for Animal Welfare

Agonal breathing

The presence of agonal breathing, spinal in origin and not rhythmic, was recorded in nine (15%) of the piglets > 3 minpost-shot. The absence of VEPs for these animals combined with an isoelectric EEG confirmed absence of cortical brain activity. Hayes et al (1988) found that concussive injury produced different effects depending on the distance from the site of impact, including depression and focal activation of brain regions. Similar movement phenomena have been attributed to activity in the spinal dorsal horn of brain-dead human patients (Urasaki et al 1992). This suggests that the complex movements shown by brain-dead humans may either reflect partial function in spinal neurons or represent the physiological potential of the intact isolated spinal cord. Spittler et al (2000) found that 10% of human brain-dead patients exhibited various spinal automatisms, while Döşemeci et al (2004) reported 13.4%, and Saposnik et al (2000) placed the figure of spontaneous and reflex movement in brain-dead patients (referred to as heart beat cadavers, HBC) higher in their study at 39%. Wijdicks (1995) describes the possible reaction of spinal neurons in response to both changes in the plasma partial pressure of CO_{2} (pCO₂) levels and pH following brain death stimulating respiratory potential. Saposnik et al (2009) reviewed historical reports of movement in HBCs from 1960 to 2007 and found reports of respiratory-like movements leading to the theory that hypoxia stimulates neurons in the spinal cord. Turmel (1991) described spinal reflex movements in an HBC manifesting once cerebrospinal shock had abated, determining that these represented isolated spinal cord physiological potential. Therefore, the possible effect of cerebrospinal shock may explain the time delay between the application of BFT and the onset of agonal breathing movements in this current study. Saposnik et al (2005) found that movement was more common in HBC with intracerebral haemorrhage (51%) than anoxic-ischaemic encephalopathy (11%), the former condition being more accurate for cases of BFT in piglets. In this study it was hypothesised that the presence of agonal gasping was spinal in origin and possibly indicative of residual partial isolated spinal neuronal activity due to changes in the plasma partial pressure of CO₂ (pCO₂) levels and pH in a brain-dead animal with a patent heartbeat.

Animal welfare implications

This experiment demonstrates that mechanical blunt-force trauma using a single-shot non-penetrating captive bolt, ie the Bock Industries Zephyr EXL, provides for an immediate stun kill in neonate piglets up to 10.9 kg liveweight and hence a humane death. This immediate loss of cortical function, combined with reproducible effects, will improve the welfare of piglets to be subjected to on-farm euthanasia due to disease, ill-thrift or commercial concerns. The technique with which the instrument is used is of utmost importance to ensure a successful and immediate death; the instrument has to be correctly positioned when the shot is fired and the piglet's head retrained against a solid surface, as shown in the text.

Conclusion

The Bock Industries Zephyr EXL has sufficient velocity and kinetic energy to stun/kill neonate piglets up to 10.9 kg live-weight, producing immediate loss of Visual Evoked Potentials in all animals with their head resting on a hard surface. Post-shot convulsions are encountered, representing enhanced spinal reflexes as would be expected in a brain-dysfunctional animal. Agonal gasping can be observed in a percentage of the animals but can be considered as indicative of partial brainstem function in an animal whose higher brain centre has been destroyed, as none of the animals demonstrated other brainstem reflexes following the application, including rhythmic breathing, corneal reflex or response to painful stimuli.

Acknowledgements

Prepared as information for Alberta Agriculture and Forestry, funding for this project was provided through Growing Forward 2, a federal-provincial-territorial initiative. The views and opinions expressed in this paper are not necessarily those of Agriculture and Agri-Food Canada or Alberta Agriculture and Forestry.

References

Agriculture and Horticulture Development Board Yearbook 2015 http://pork.ahdb.org.uk/media/271531/k11363ahdb-pork-yearbook-for-digital-delivery-aw.pdf

Armstead WM 1999 Cerebral hemodynamics after traumatic injury of immature brain. *Experimental and Toxicologic Pathology 51*: 137-142. https://doi.org/10.1016/S0940-2993(99)80087-6

Armstead WM and Kurth CD 1994 Different cerebral hemodynamic responses following fluid percussion brain injury in the newborn and juvenile pig. *Journal of Neurotrauma 11(5)*: 487-497. https://doi.org/10.1089/neu.1994.11.487

Baumer T, Passalacqua N, Powell B, Newberry W, Smith W, Fenton T and Haut R 2010 Age dependent fracture characteristics of rigid and compliant surface impacts on the infant skull: A porcine model. *Journal of Forensic Science 55(4)*: 993-997. https://doi.org/10.1111/j.1556-4029.2010.01391.x

Casey-Trott TM, Millman ST, Turner PV, Nykamp SG, Lawless PC and Widowski TM 2014 Effectiveness of a non-penetrating captive bolt for euthanasia of 3Kg to 9Kg pigs. *Journal of Animal Science* 92: 5166-5174. https://doi.org/10.2527/jas.2014-7980 Casey-Trott TM, Millman ST, Turner PV, Nykamp SG and Widowski TM 2013 Effectiveness of a non-penetrating captive bolt for euthanasia of piglets less than 3 d of age. *Journal of Animal Science* 91: 5477-5484. https://doi.org/10.2527/jas.2013-6320

Daly CC, Gregory NG and Wotton SB 1987 Captive bolt stunning of cattle: Effects on brain function and role of bolt velocity. British Veterinary Journal 143: 574. https://doi.org/ 10.1016/0007-1935(87)90049-2

Döşemeci L, Cengiz M, Yılmaz M and Ramazanoğlu A 2004 Frequency of spinal reflex movements in brain-dead patients. *Transplantation Proceedings* 36: 17-19. https://doi.org/ 10.1016/j.transproceed.2003.11.049 Duhaime AC, Margulies SS, Durham SR, O'Rourke MM, Golden JA, Marwaha S and Raghupathi R 2000 Maturationdependent response of the piglet brain to scaled cortical impact. *Journal of Neurosurgery* 93: 455-462. https://doi.org/10.317 1/jns.2000.93.3.0455 Finnie JW 1993 Brain damage caused by a captive bolt pistol.

Journal of Comparative Pathology 109: 253-258. https://doi.org /10.1016/S0021-9975(08)80250-2

Finnie JW, Manavis J, Summersides GE and Blumbergs PC 2003 Brain damage in pigs produced by impact with a nonpenetrating captive bolt pistol. *Australian Veterinary Journal 81(3)*: 153-155. https://doi.org/10.1111/j.1751-0813.2003.tb11078.x

Gregory NG 1993 Euthanasia. The assessment of welfare and scientific aspects. World Congress on Alternatives and Animal Use in the Life Sciences, Volume 26. Baltimore, USA

Gregory NG 1998 Animal Welfare and Meat Science. CABI Publishing: Wallingford, UK

Gregory NG and Wotton SB 1983 Studies on the central nervous system: visually evoked cortical responses in sheep. Research in Veterinary Science 34: 315-319

Gregory NG and Wotton SB 1984 Sheep slaughtering procedures 2. Time to loss of brain responsiveness after exsanguination or cardiac arrest. *British Veterinary Journal 140*: 354-360. https://doi.org/10.1016/0007-1935(84)90126-X

Guerit MJ 1999 Medical technology assessment: EEG and evoked potentials in the intensive care unit. *Clinical Neurophysiology* 29: 301-317. https://doi.org/10.1016/S0987-7053(99)90044-8

Hayes RL, Katayama Y, Young HF and Dunbar JG 1988 Coma associated with flaccidity produced by fluid-percussion concussion in the cat part 1. Is it due to depression of activity within the brainstem reticular formation? *Brain Injury* 2: 31-49. https://doi.org/10.3109/02699058809150930

Matthis JS 2004 Selected employee attributes and perceptions regarding methods and animal welfare concerns associated with swine euthanasia. PhD Dissertation, North Carolina State University, Raleigh, NC, USA Millman ST 2010 Mechanical euthanasia methods – process and physiology. AASV Annual Meeting: Implementing Knowledge pp 443-446. 6-9 March 2010, Nebraska, USA

Mort M, Convery I, Baxter J and Bailey C 2008 Animal disease and human trauma: The psychosocial implications of the 2001 UK foot and mouth disease disaster. *Journal of Applied Animal Welfare* 11(2): 133-148. https://doi.org/10.1080/10888 700801925984

Ommaya AK and Gennarelli TA 1974 Cerebral concussion and traumatic unconsciousness. *Brain* 97: 633-654. https://doi.org /10.1093/brain/97.1.633

Ommaya AK, Grub RL and Naumann RA 1971 Coup and contra-coup injury: observations on the mechanics of visible brain injuries in the rhesus monkey. *Journal of Neurosurgery* 35: 503-516. https://doi.org/10.3171/jns.1971.35.5.0503

Powell BJ, Baumer TG, Passalacqua NV, Wagner CD, Haut RC, Fenton TW and Yang KH 2012 A Forensic Pathology Tool to Predict Pediatric Skull Fracture Patterns. Final Technical Report, US Department of Justice Award Number 2007-DN-BX-K196, Document Number 240683. https://www.ncjrs.gov /pdffiles1/nij/grants/240683.pdf

Saposnik G, Basile VS and Young GB 2009 Movements in brain death: A systematic review. *Canadian Journal of Neurological Science* 36: 154-160. https://doi.org/10.1017/S031716710000651X

Saposnik G, Bueri JA, Mauriño J, Saizar R and Garretto NS 2000 Spontaneous and reflex movements in brain death. *American Academy of Neurology 54*: 221. https://doi.org/10.121 2/WNL.54.1.221

Saposnik G, Mauriño J, Saizar R and Bueri JA 2005 Spontaneous and reflex movements in 107 patients with brain death 2005. The American Journal of Medicine 118: 311-314. https://doi.org/10.1016/j.amjmed.2004.09.013

Sharp TM, McLeod SR, Leggett KEA and Gibson TJ 2014 Evaluation of a spring-powered captive bolt gun for killing kangaroo pouch young. *Wildlife Research* 41: 623-632. https://doi.org/10.1071/WR14094

Shaw NA 2002 The neurophysiology of concussion. Progress in Neurobiology 67: 281-344. https://doi.org/10.1016/S0301-0082(02)00018-7

Somjen G 2001 Mechanisms of spreading depression and hypoxic spreading depression-like depolarization. *Physiological Reviews* 81(3): 1065-1096

Spittler JF, Wortmann D, von Düring M and Gehlen W 2000 Phenomenlogical diversity of spinal reflexes in brain death. *European Journal of Neurology* 7: 315-321. https://doi.org/10.1046/j.1468-1331.2000.00062.x

Turmel A, Roux A and Bojanowski MW. 1991 Spinal man after declaration of brain death. *Neurosurgery* 28: 298-301. https://doi.org/10.1227/00006123-199102000-00021

Urasaki E, Tokimura T, Kumai J, Wada S and Yokota A 1992 Preserved spinal dorsal horn potentials in a brain dead patient with Lazarus' sign. Case report. *Journal of Neurosurgery* 76: 710-713. https://doi.org/10.3171/jns.1992.76.4.0710

Whiting TL and Marion CR 2011 Perpetration-induced traumatic stress: A risk for veterinarians involved in the destruction of healthy animals. *Canadian Veterinary Journal 52*: 794-796

Whiting TL, Steele GG, Wamnes S and Green C 2011 Evaluation of methods of rapid mass killing of segregated early weaned piglets. *Canadian Veterinary Journal 52(9)*: 753-758

Wijdicks EFM 1995 Determining brain death in adults. *Neurology* 45: 1003-1011. https://doi.org/10.1212/WNL.45.5.1003

Wotton SB and Gregory NG 1986 Pig slaughtering procedures: time to loss of brain responsiveness after exanguination or cardiac arrest. Research in Veterinary Science 40: 148-151