

## Humane euthanasia of neonates II: field study of the effectiveness of the Zephyr EXL non-penetrating captive-bolt system for euthanasia of newborn piglets

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### Abstract

A previous study demonstrated the effectiveness of the Bock Industries Zephyr EXL non-penetrating captive bolt, using the abolition of visual-evoked potentials as a determination of brain death, in piglets in a laboratory. A second trial, reported here, involved the field-testing of this device, on-farm, in a commercial setting. Two hundred and seven piglets (mean [ $\pm$  SEM] dead weight: 1.86 [ $\pm$  0.74] kg) requiring dispatch under the farm's protocols were euthanased with the device and demonstrated immediate loss of consciousness, subjectively assessed by behavioural signs and no recovery. Post mortem examination of the heads was undertaken confirming massive traumatic damage to the cerebrum with associated haemorrhage and bone plate shards forced down to the level of the corpus callosum in the majority of cases. A further trial of 106 piglets demonstrated that under commercial production conditions it took less than 7 s to select, place and euthanase a piglet using the device. One hundred percent of animals in the study were immediately killed. Given this complete kill rate and the sample size of the study, a statistical 95% confidence interval provides a maximum percentage of animals that would not immediately be stunned/killed, by this mechanical non-penetrating captive-bolt system, to be at most 1.2% and at least 0%. The results here, combined with the previous study, allow recommendation of the Bock Industries Zephyr EXL as being suitable as a single application euthanasia device for piglets up to 10.9 kg liveweight.

**Keywords:** animal welfare, captive bolt, euthanasia, mechanical stunning, piglet, Zephyr EXL

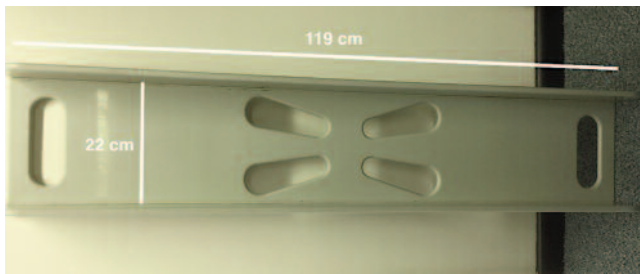
### Introduction

On-farm casualty killing, or the killing of surplus animals, is traditionally performed by manual blunt-force trauma which is carried out by administering a blow to the head either with a hammer or similar heavy instrument or, by swinging the young animal against the floor or a wall (Farm Animal Welfare Committee [FAWC] 2017). Although this method is widely used, it is heavily dependent on the strength and skill of the stockperson and, consequently, the probability of achieving an immediate and humane kill in all cases is low. Furthermore, a lack of proper training and human error can result in pain and distress for the animal. It is also a method of killing that is aesthetically unpleasant for both the operator and any bystanders. Council Regulation (EC) 1099/2009 of 24 September 2009 on the Protection of Animals at the Time of Killing, Annex 1 limits the use of a manual percussive blow to the head to piglets  $\leq$  5 kg live weight, stating that this method shall not be used as a routine method but only where there are no other methods available for stunning and no more than seventy animals per operative, per day, may be killed by this method.

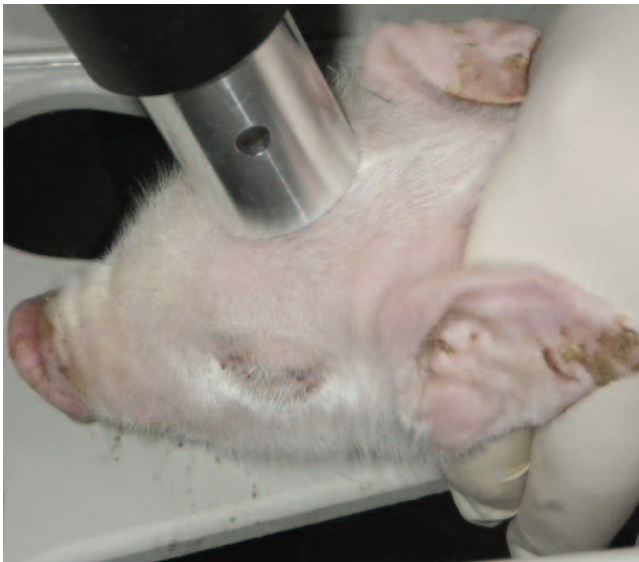
Earlier field studies using the lower powered Zephyr E (bolt energy = 20 J) employed a three-application method (two in

rapid succession on the frontal bone followed by a third at the back of the skull behind one ear) (Casey-Trott *et al* 2013) or, a two-application method (Casey-Trott *et al* 2014) for the euthanasia of piglets. Following these studies, the manufacturer, Bock Industries PA, USA developed the Zephyr EXL, which delivers more power (bolt energy = 27.7 cf 20 J) to negate the requirement for repeat application of the device to the animal. A previous study examined the effectiveness of the higher powered Zephyr EXL non-penetrating captive-bolt system, for the euthanasia of newborn and weaned piglets up to 10.9 kg liveweight (Grist *et al* 2017). This study, conducted under laboratory conditions, demonstrated that a single application positioned on the frontal-parietal bone abolished visual-evoked potentials immediately in all the piglets.

The American Veterinary Medical Association (AVMA 2013), amongst others, encourages those using manual blunt-force trauma as a euthanasia method to seek alternative techniques, for example, they recommended that a mechanical percussive blow to the head can be used for piglets up to three weeks of age. As such, this non-penetrating captive bolt mechanical blunt force trauma (MBFT) device, ie the Zephyr EXL, can be considered to comply

**Figure 1**

Polypropylene Bock Industries restrainer used in trials.

**Figure 2**

Application position — body restrained with free hand.

**Table 1 Subjective scoring system used to assess post-stun/kill movement based on level of post-application spinal reflex activity, ranging from 0 (no clonic activity post-stun) to 3 (severe uncontrolled physical movement [padding]).**

Score	Descriptor	Description
0	No activity	Very little convulsive movement
1	Mild activity	Some physical movement of limbs (padding) that is manageable
2	Moderate activity	Considerable physical movement of the limbs
3	Severe	Severe physical movement (padding of limbs)

with the US Humane Slaughter Act Section 1902 that requires that an animal is rendered insensible to pain by a single blow and Council Regulation (EC) 1099/2009 of 24 September 2009 on the Protection of Animals at the Time of Killing, Annex 1. Therefore, the use of mechanical non-penetrative captive guns to both stun and kill neonate piglets offers a more reproducibly humane method of casualty killing on-farm.

This second stage of the assessment of the Zephyr EXL involved a field trial that was conducted in two phases: Phase one assessed the dispatch of 207 piglets using the Zephyr EXL with a single application in the frontal-parietal area; Phase two was the dispatch of 106 piglets at operational speed, the second phase being conducted once confidence of the outcome was gained from Phase one. The Phase two trial was a practical, on-farm confidence assessment.

## Materials and methods

### Study animals and procedures

The overall study was carried out with the approval of the University of Bristol's Ethical Review Committee.

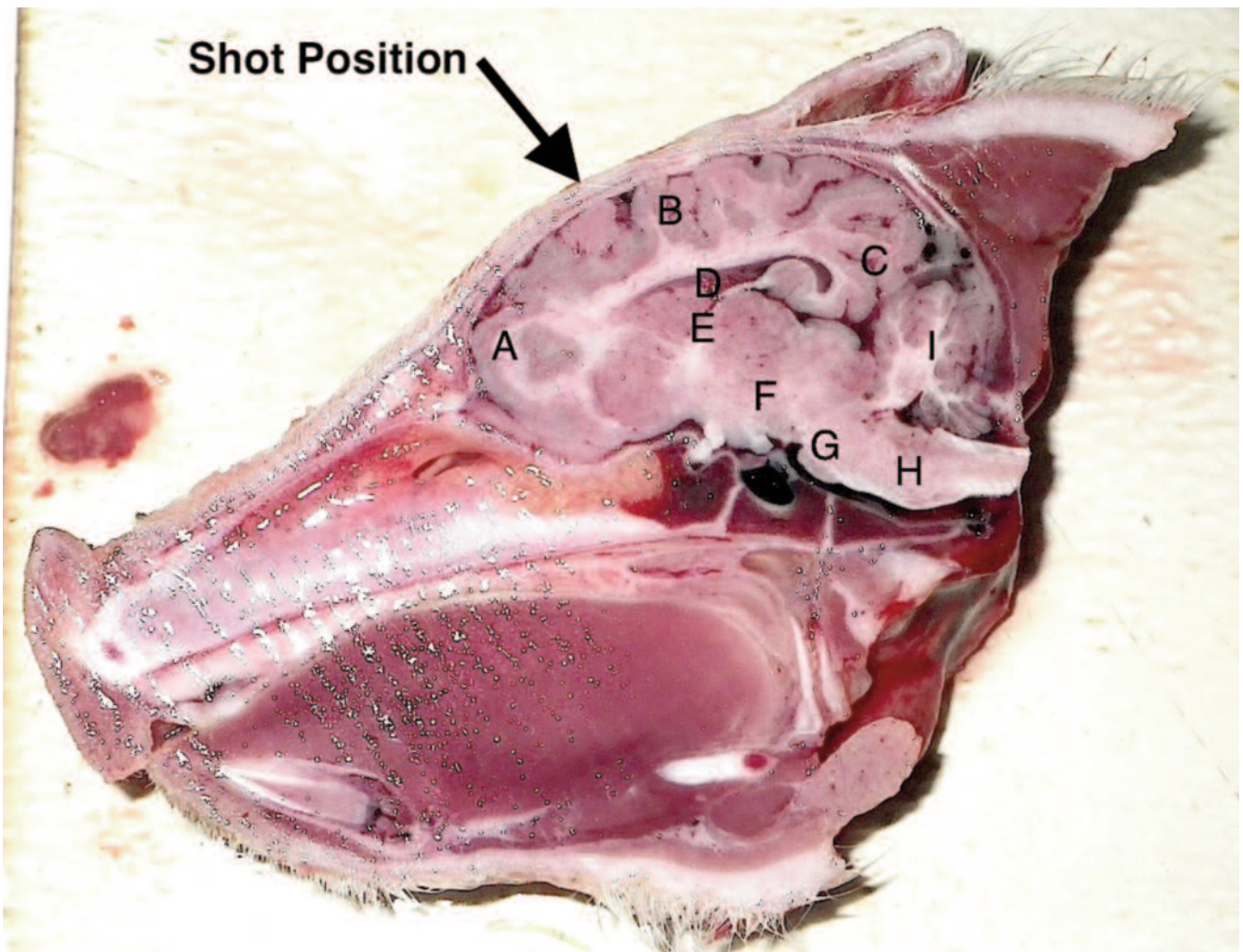
#### Phase one: Field trial

Compromised piglets ( $n = 207$ ; mean [ $\pm$  SEM] weight:  $1.86 \pm 0.74$  kg) were collected and brought to the shooting point separate to the farrowing area in a  $\sim 15,000$ -sow commercial unit in Texas, USA. All the piglets were selected by experienced farm personnel as requiring euthanasia following the normal criteria operated in the commercial unit. Each piglet was individually restrained on a polypropylene swine restraint assembly (Bock Industries; Figure 1) having its legs placed through the holes in the assembly by an experienced researcher (SBW) whilst being loosely held with one hand and the device applied once in the parietal position (Figure 2) with a Zephyr EXL (Bock Industries, PA, USA, Gun number 722) powered at 120 psi by a Hitachi EC510 air compressor (Hitachi Koki USA Ltd, Georgia, USA). It has been reported (Grist *et al* 2017) that it is important that, as well as the use of the correct application position, the head of the piglet must be held firmly against the restrainer surface.

Following the application each piglet was examined by the researcher (SBW) for behavioural signs of brain dysfunction including loss of rhythmic breathing, loss of palpebral and corneal reflex and non-responsiveness to nose prick with a hypodermic needle. The presence of a heartbeat was also assessed by the researcher (SBW) by auscultation. Movement post-application was assessed subjectively based on the descriptors given in Table 1 whilst the animal was still in restraint. All measurements were continued for a minimum of 3 min post-application and all findings were recorded by a second technician. Recovery of rhythmic breathing did not occur in these animals during the assessment period (3 min), which would indicate severe irrecoverable damage to the brainstem, the control centre for breathing (Hewitt 2000).

Once death was verified (no brainstem reflexes and no breathing for  $> 3$  min), the piglet was numbered on the ear with an indelible marker, removed from restraint and

Figure 3



Sagittal section of a piglet head (intact found dead) illustrating the areas examined for macroscopic damage showing A) frontal cerebrum, B) parietal cerebrum, C) occipital cerebrum, D) lateral ventricle. These areas were scored on the basis of 0 = no damage, 1 = 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 haemorrhage.

weighed to the nearest 0.25 kg on a 44 lb stainless steel dial scale (Item No 755105 Gandermountain, USA). The head was removed, placed in a resealable zipperlock bag with the corresponding piglet number repeated on the outside. The bagged heads were placed in a freezer for 24 h for subsequent sectioning and examination. Freezing being undertaken to facilitate sectioning on the medial plane without disruption of macroscopic lesions during cutting.

#### Post mortem examination of the head

Each head was removed from the bag once the number had been noted, the ear number was checked to ensure correlation. The head was split on the sagittal plane using an electric band saw (Craftsman, USA) and both sides were photographed on the medial plane with a Pentax WG-1 digital camera (Pentax Ricoh Imaging Company Ltd, Japan) and post mortem findings recorded. Two researchers agreed on the macroscopic brain lesions, blind to the

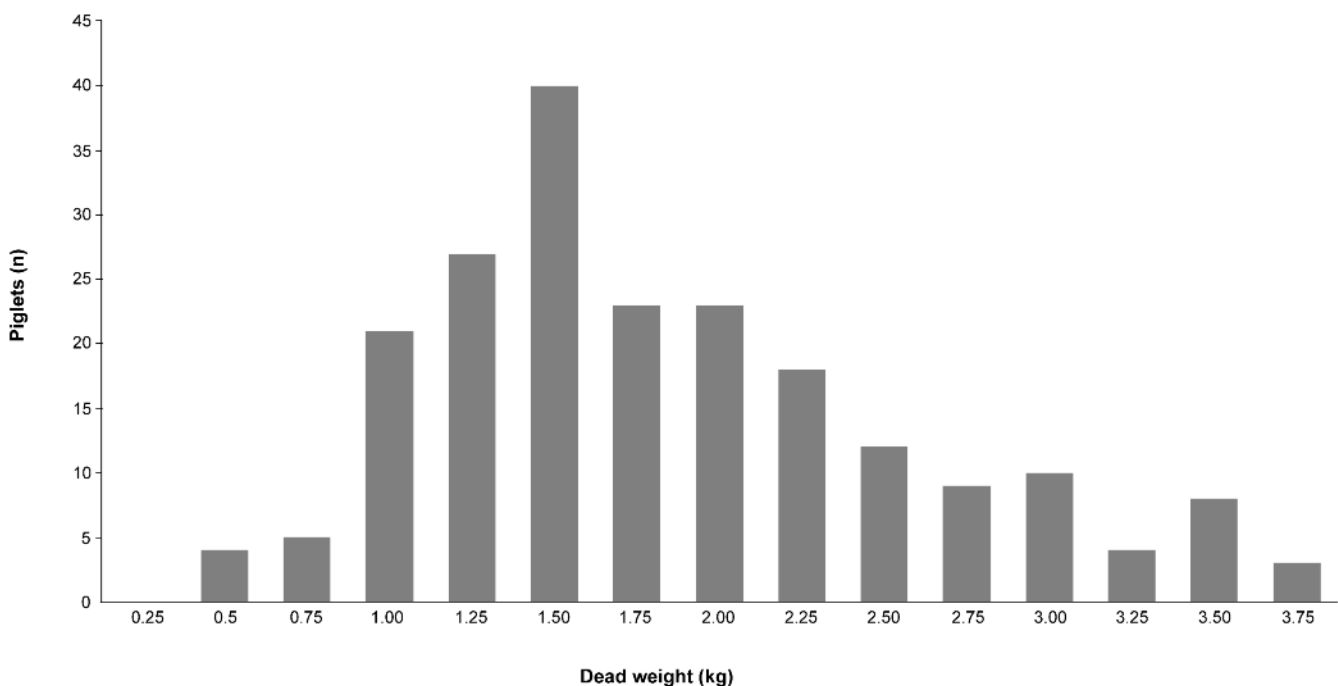
weight ranges and piglet numbers, utilising a subjective scale adapted from Sharp *et al* (2014) where 0 = no damage, 1 = slight deformation, 2 = moderate deformation and 3 = severe deformation of the area. The areas examined for macroscopic damage were the frontal, parietal and occipital cerebrum, including the structure of the lateral ventricle as detailed in Figure 3.

#### Phase two: Practical on-farm application trial — assessment of the speed of operation

Piglets (n = 106), selected by the farm staff on the same farm as Phase one, as requiring euthanasia following their normal selection protocols, were delivered to the study area, separate to the farrowing area, in wheeled containers. Each piglet, of similar age and weight to Phase 1, selected at random, within ten groups of between ten and 13 animals (Table 2), was removed from the container by the researcher (SBW), placed on the restraint device and the device

**Table 2** Time taken to perform euthanasia on groups of piglets using the Zephyr EXL non-penetrating captive bolt.

Number of piglets in group	Time taken per group (s)	Average time per piglet (s)
13	108	8.31
13	108	9.31
10	61	6.10
10	55	5.50
10	56	5.60
10	67	6.70
10	57	5.70
10	64	6.40
10	64	6.40
10	55	5.50
<b>n = 106</b>	<b>695</b>	<b>Mean 6.45 s per piglet</b>

**Figure 4**

Weight range distribution of piglets in trial (n = 207; weight 1.86 [ $\pm$  0.74] kg).

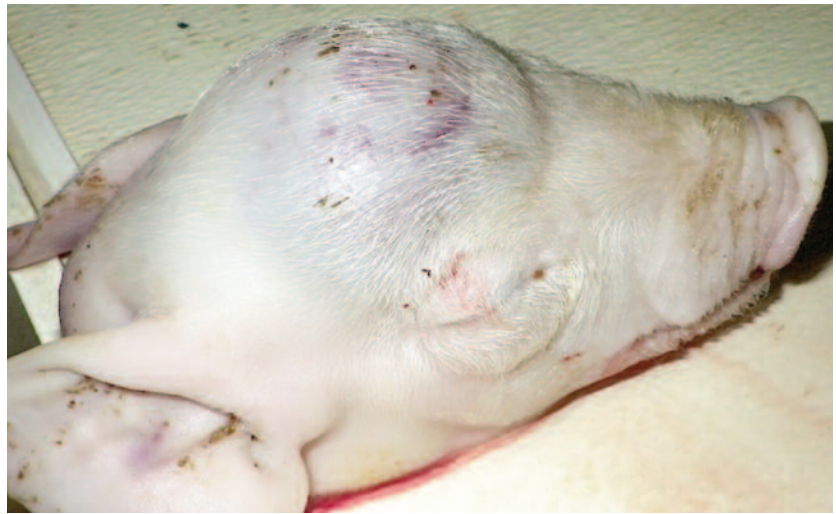
applied once by the researcher in the frontal-parietal position before being placed in a second container. The time taken to stun/kill each batch of piglets from placing each piglet, applying the method and removing the piglet, was recorded and the effectiveness of the operation on the piglets in the second container was assessed by a second experienced researcher using the behavioural indices of brain dysfunction described above, death being verified by no rhythmic breathing movements for  $\geq$  3 min.

#### Statistical analysis

Statistically, 100% efficiency can never be absolutely proven, there will always be some small margin for error, however large the study. However, a sample size of 200 was, in practice, a reasonable figure to demonstrate the degree of efficacy of the method, this initial figure was increased to > 300 (n = 313) by the addition of data from Phase two of the trial. A sample of this size would give a 95% confidence interval, should 100% of 313 animals be

Figure 5

Characteristic haematoma associated with impact point of device



effectively stun/killed, that the very maximum possible percentage of animals not immediately stunned/killed in normal use, and therefore requiring a second application of the device would be, at most, no more than 1.2% (Wilson's Method in Altman *et al* 2000). In addition to presenting the confidence intervals for single sample estimates, the correlations between relevant variables were assessed using Spearman's rho ( $r_s$ ). The post mortem macroscopic brain lesion results were also tested for an overall linear correlation between individual piglet weight and the score for each of the four areas using a two-tailed Spearman's rank correlation test (Spearman's rho [ $r_s$ ]). All statistical analyses were carried out using IBM SPSS Statistics (v23).

## Results

### Phase one: Field trial

#### Piglet weight

The distribution of piglet dead weights is shown in Figure 4, with a range from 0.25 to 3.75 kg (weight  $1.86 \pm 0.74$  kg). The ages of the piglets ranged from newborn to 15 days with an average of 4.43 days.

#### Behavioural indices of brain death

One hundred percent of the piglets demonstrated immediate cessation of brainstem reflexes ( $n = 207$ ; 95% CI for percentage immediately killed = 0 to 1.8% [Wilson's method]), including rhythmic breathing, palpebral reflex, corneal reflex and pain stimuli response. One piglet received a second application of the device in the poll position as a precaution as it demonstrated some agonal gasping movements at 10 min post-application, however the piglet demonstrated no other brainstem reflexes. The piglet stopped agonal breathing immediately following the second application.

#### Animal movement

Correlation analysis (Spearman's rho) demonstrated a significant correlation between (i) movement scores and weight ( $r_s = 0.611$ ,  $n = 207$ ;  $P \leq 0.001$ ) demonstrating that higher movement scores were associated with heavier piglets, (ii) weight and end of movement time ( $r_s = -0.364$ ,  $n = 198$ ;  $P \leq 0.001$ ) demonstrating that the larger weight piglets cease movements earlier than lighter piglets and (iii) weight/age and end of movement time ( $r_s = -0.405$ ,  $n = 207$ ;  $P \leq 0.001$ ) demonstrating that higher movements are associated with younger piglets.

#### Agonal gasping

Thirty-four (16.43%) of the piglets displayed intermittent agonal gasping movements post-application. None of these animals demonstrated any brainstem or cranial reflexes and all had a 'normal' heartbeat immediately post-stun. There was no significant correlation between the onset of agonal breathing and age ( $r_s = -0.065$ ,  $n = 34$ ;  $P = 0.716$  [two-tailed]) or weight ( $r_s = 0.143$ ,  $n = 34$ ;  $P = 0.4211$  [two-tailed]).

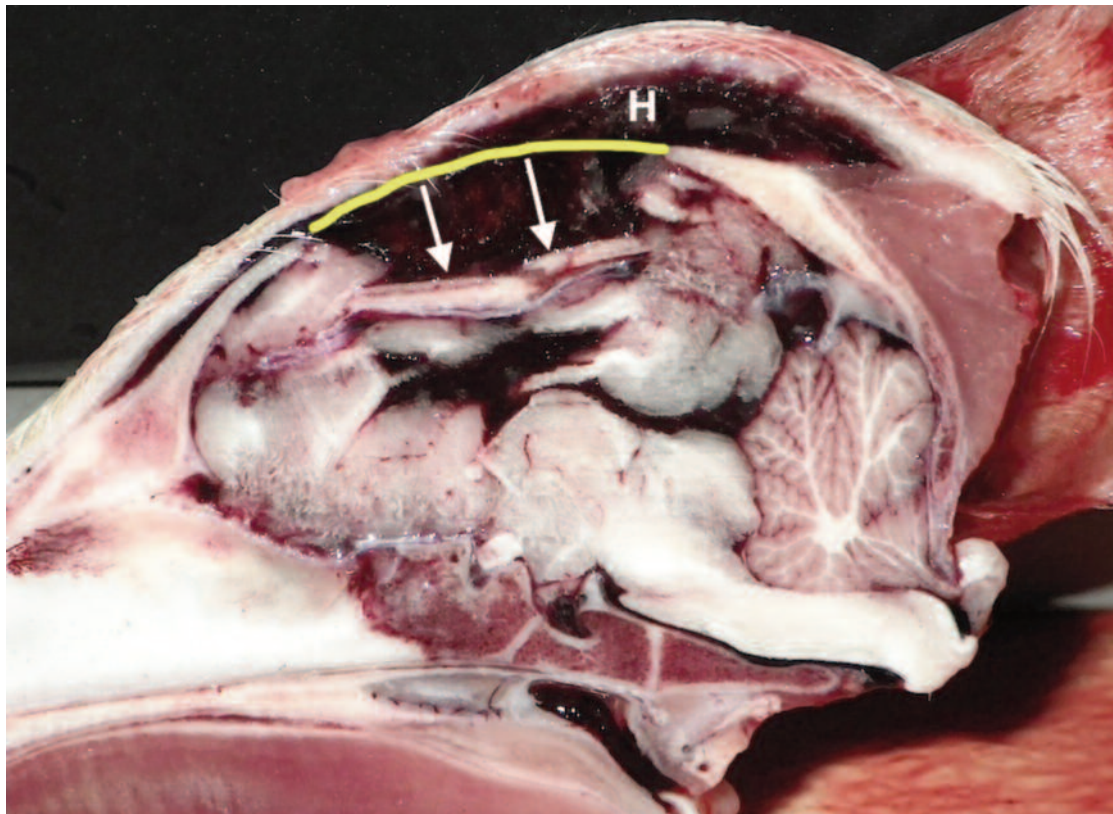
#### Haematoma and bleeding

Nasal haemorrhages were recorded with 158 piglets (76.33%; 95% CI = 70.1 to 81.6%) and laceration to one side of the impact point was recorded with 31 piglets (14.98%; 95% CI = 10.8 to 20.5%), the piglets with the latter condition were the only experimental animals that did not develop a large haematoma over the impact point (Figure 5).

#### Post mortem

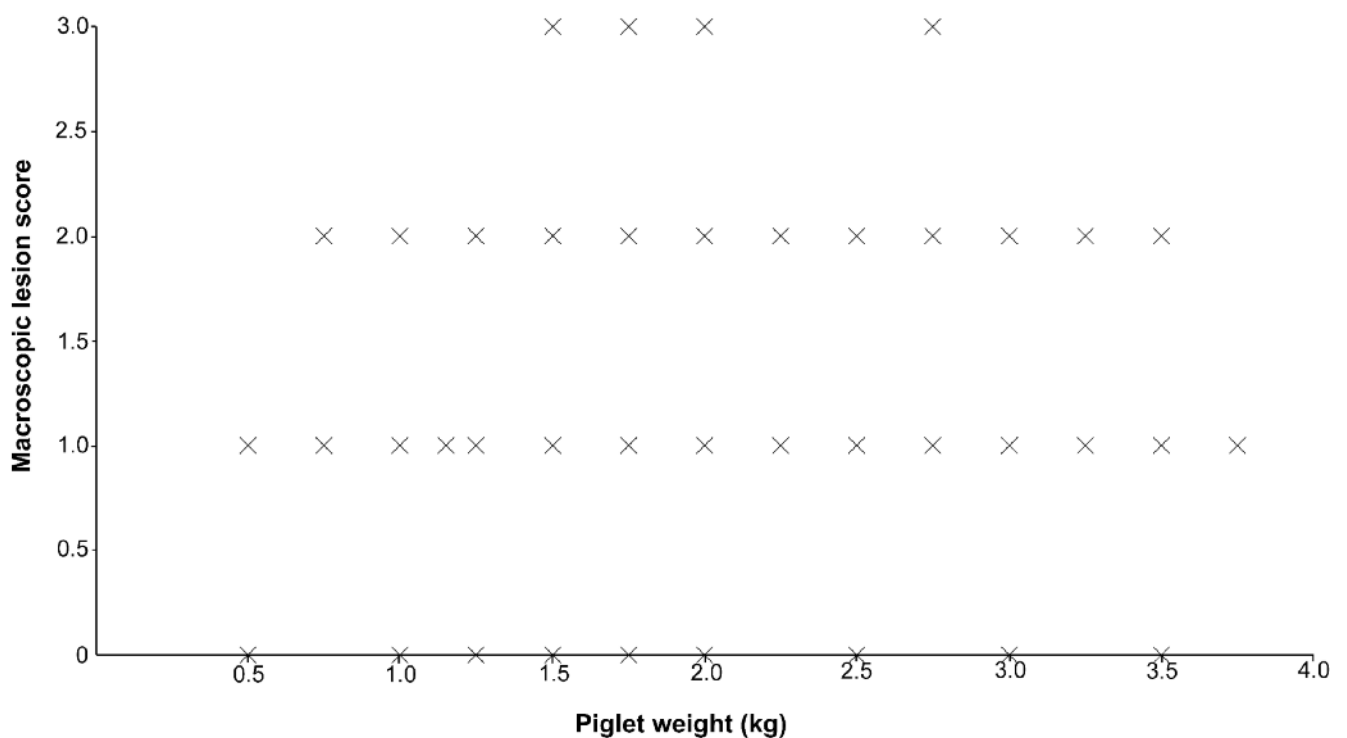
The main post mortem finding was a depressed fracture of the skull with a concurrent subdural haematoma with parts of the occipital and parietal lobes prolapsed through the fracture. Fracture of the parietal plate was a common finding with bone shards forced into the medial dorsal cerebrum resulting in crushing of the frontal lobe of the cerebrum. The structure of the corpus callosum was

Figure 6



Example of post-application trauma, medial sagittal section Piglet 17. Haematoma (H) evident, yellow line denotes approximate curve of cranial cavity in the live animal, the arrows demonstrating the displacement of bone shards into the brain.

Figure 7



Graph of macroscopic lesion score of the frontal cerebrum against piglet weight. Subjective brain macroscopic lesion scores (0 = no damage, 1 = slight deformation, 2 = moderate deformation and 3 = severe deformation of the area) for the frontal cerebrum gave a positive correlation with piglet weight that was very statistically significant (Spearman's rho = 0.185, n = 207; P = 0.008).

generally severely compromised and parenchymal haemorrhages were evident within the thalamus, frontal parietal and occipital lobes of the cerebrum. Haemorrhages were encountered throughout the cranial cavity (Figure 6).

#### Macroscopic lesion scoring

Macroscopic lesion scores for the parietal cerebrum showed no correlation (Spearman's  $\rho = 0.082$ ,  $n = 6,206$ ;  $P = 0.243$ ) with piglet weight and neither did occipital cerebrum lesion scores (Spearman's  $\rho = 0.006$ ,  $n = 206$ ;  $P = 0.0.934$ ) or lateral ventricle lesion scores (Spearman's  $\rho = -0.054$ ,  $n = 206$ ;  $P = 0.437$ ). Figure 7 demonstrates the positive correlation between the macroscopic brain lesion score of the frontal cerebrum and piglet weight.

#### Phase two: Practical on-farm application trial

All the piglets showed immediate loss of sensibility and no positive brainstem reflexes or a response to a nose prick with a hypodermic needle ( $n = 106$ ; 95% CI for percentage immediately killed = 0 to 3.5%). The mean time taken to pick up a piglet, place it in the restrainer, shoot and remove it from the restrainer was 6.45 s (Table 2).

Following successful completion of experimental Phases one and two, the study team undertook on-farm training of farm staff to explain the correct use of the instrument and the scientific basis of non-penetrating captive bolt (NPCB) operation, and most importantly the physical and physiological signs that should be expected in a properly stunned/killed piglet.

#### Discussion

The Zephyr EXL powered at 120 psi provided for immediate loss of consciousness and brain death in all 207 piglets in a commercial setting with a single application of the device to the frontal-parietal position and with the head resting against a hard surface (Bock Industries restrainer; Figure 1). This field trial validates the findings of the laboratory assessment of the effectiveness of the Zephyr EXL in which visual-evoked potentials were immediately lost in all piglets with the device applied in the same manner (Grist *et al* 2017). The design and reporting of this and the previous study (Grist *et al* 2017) was guided by the published European Food Safety Authority (EFSA) guide (EFSA 2013) in order to ensure that the information can be used by EFSA to assess whether the use of a non-penetrating captive bolt can be used as a killing method for neonate piglets. Combining the figures from the two on-farm trials ( $n = 313$ ), the upper confidence limit (Wilson's method) is reduced so that, based on this relatively small-sized trial, the very maximum percentage of animals not immediately stunned/killed and requiring a second application would be no more than 1.2% when used commercially.

As with the previous laboratory assessment a percentage of animals demonstrated agonal gasping (not rhythmic breathing) post-application (16.43% in the field trial cf 15% in the laboratory). This was previously theorised to be indicative of residual partial isolated spinal neuronal

activity due to changes in the plasma partial pressure of  $\text{CO}_2$  ( $p\text{CO}_2$ ) levels and pH in a brain-dead animal with a 'normal' post-stun/kill heartbeat (Wijdicks 1995). St John (2009) describes the activation of latent pacemaker mechanisms following hypoxia, ischaemia or removal of the influence of the pons and rostral medulla, Terlouw *et al* (2016) stated that gasping precedes death and reflects dysfunction of brain areas including centres within the pons and medulla.

The macroscopic brain lesion and movement score results differed from the laboratory experiment reported by Grist *et al* (2017) which found a reduction in macroscopic brain lesions with increased piglet weight. It is presumed that this is due to various factors including the smaller weight range of the animals in this field trial (0.5 to 3.75 kg cf 1.0 to 10.9 kg), and the fact that the field trial piglets were euthanased for ill health and under performance (with possible lack of skull development) as opposed to the laboratory trials which purposely used healthy animals to give a conservative assessment of the effectiveness of the Bock Industries Zephyr EXL on piglets of a given weight range. There was no correlation found between the macroscopic brain lesion scores and either movement score or time to loss of movement.

During the field trial, the farm workers expressed some concern over the post-application movements and this concern correlated with the findings of previous studies (Matthis 2004; Gemus-Benjamin 2015). This concern abated once the physical characteristics of post stun/kill spinal reflexes were explained during a training seminar where the absence of brainstem reflexes was practically demonstrated by the researchers. Movement following brain disruption has long been hypothesised as being due to spinal rhythm generators (Pearson *et al* 1998; Guertin 2009) and does not relate to consciousness; this has been demonstrated by Terlouw *et al* (2015) who recorded movement in stunned cattle after isolation of the spinal cord by post-stun severance between the foramen magnum and the first cervical vertebra. The level of post-brain death movement encountered in the piglets corresponds to levels found in human patients that in various studies ranged from 10% spinal automatisms (Spittler 2000) to as high as 39% (Saposnik 2000).

#### Animal welfare implications

This mechanical blunt force trauma device (Bock Industries Zephyr EXL) provides an immediate, single application, stun-kill of neonate piglets that may require dispatch for any purpose including ill health, disease control, mercy or production efficiencies. The device also has a low deviation (< 1%) of velocity with each firing (J Lines, personal communication 2015). It is important that the behavioural signs of a proper stun/kill application are explained to operators and that clonic activity will occur but is a sign of a successful application.

## Conclusion

This field trial, in conjunction with laboratory trials (Grist et al 2017), lead to the conclusion that the Bock Industries Zephyr EXL is effective in producing a stun/kill in neonate piglets (up to 10.9 kg) with a single application placed on the top of the head at 120 psi with the head resting on a solid surface; as such, it represents a viable alternative to manual blunt-force trauma as a method of piglet dispatch.

## Acknowledgements

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