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Evaluation of the CASH Dispatch Kit combined with alternative shot placement landmarks as a single-step euthanasia method for cattle of various ages

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Abstract

Humane euthanasia of cattle under field conditions presents special challenges for veterinarians and producers. The purpose of this study was to evaluate the effectiveness of the CASH Dispatch Kit captive-bolt system combined with improved shot placement landmarks as a single-step euthanasia method for cattle. Cattle destined for euthanasia for reasons unrelated to the study were utilised. Adult (> 2 years), young (6–24 months) and neonatal (< 1 month) cattle each received a single shot from the CASH penetrating captive-bolt pistol. An additional group of neonatal animals was shot with a non-penetrating muzzle attachment. The shot was placed on midline halfway between the top of the poll and an imaginary line connecting the lateral canthus of each eye. Following the shot, the animals were immediately assessed for loss of consciousness based upon: i) immediate collapse (if standing); ii) loss of eye reflexes with a centered, dilated pupil; iii) lack of co-ordinated respiration; iv) lack of vocalisation; and v) lack of a righting reflex. Lack of consciousness and heartbeat were assessed at 1-min intervals until cardiac arrest. All animals were adequately stunned by a single shot. Euthanasia via a single shot was successful in 28/31, 17/19, 8/10, and 9/10 adult, young, neonate (penetrating) and neonate (non-penetrating) animals, respectively. Reasons for failure included return of co-ordinated respiration and prolonged time until cardiac arrest. A single shot from the CASH Dispatch captive-bolt system will humanely euthanase most animals. However, the results of this study indicate that application of a follow-up step to ensure death is still needed in certain instances.

Keywords: *animal welfare, brainstem, captive bolt, cattle, euthanasia, single step*

Introduction

Humane euthanasia of cattle presents special challenges for veterinarians and livestock producers. Available approved methods are limited to barbiturate overdose and physical disruption of the brain via gunshot or captive-bolt device (American Veterinary Medical Association [AVMA] 2013). For on-farm use, gunshot or captive bolt are the most applicable methods. Barbiturate overdose is limited to use by a veterinarian and has the potential to result in dangerous chemical residues if carcases are not disposed of properly. It also requires excellent restraint and close contact with the animal which make it difficult to safely apply in many situations. Gunshot can be effective and is generally readily available in most livestock-producing areas in the United States. However, gunshot requires skilled operators and carries significant safety concerns that must be addressed. Also, gunshot may not be available in some areas due to legal restrictions. Captive-bolt devices are readily available, have fewer legal restraints than firearms and can be effective in the hands of trained personnel. Safety concerns do exist, but they are generally less than those associated with a firearm and free bullet.

Numerous published studies have investigated the effectiveness of penetrating captive-bolt devices for stunning of animals prior to slaughter. In a slaughter setting, animals are typically exsanguinated within a few seconds of the captive-bolt shot. Very few studies have been published specifically investigating the use of a penetrating captive bolt for the purpose of euthanasia using live animals when a secondary step, such as exsanguination, is not performed immediately after rendering the animal unconscious with the captive bolt.

The initial goal in euthanasia is to rapidly induce unconsciousness so that an animal is insensible to noxious stimuli or stress (AVMA 2013) with death occurring rapidly after loss of consciousness. Consciousness is controlled by both the cerebral cortex and brainstem (Gregory & Shaw 2000).

More specifically, the state of consciousness is controlled by the ascending reticular activating system in the brainstem (de Lahunta & Glass 2009; Smith *et al* 2009). Specifically targeting the brainstem with a penetrating captive bolt will also disrupt the cerebral cortex and will increase the probability of immediate and permanent loss of consciousness.

Unconsciousness can be instantaneously induced by application of sufficient concussive forces to the brain (Gregory & Shaw 2000; Shaw 2002) and it is thought that concussive forces of the bolt striking the skull are responsible for the immediate loss of consciousness following captive-bolt shot (Daly & Whittington 1989). In addition to concussive forces, the application of a captive bolt results in physical disruption of brain tissue by the bolt and bone fragments, acceleration and/or rotation of the brain within the skull, and intra-cranial haemorrhage. Each of the factors can impact the depth and permanence of unconsciousness (Finnie & Blumbergs 2002).

Captive-bolt devices can readily induce unconsciousness. In order to pass welfare audits in the United States, beef slaughter plants must be able to induce immediate unconsciousness in a minimum of 95% of the animals with a single captive-bolt shot (Grandin 2010). In a typical slaughter setting, exsanguination performed shortly after captive-bolt shot is the ultimate cause of death. However, a potential limitation to the use of captive bolts for euthanasia is that the loss of consciousness may not always be permanent (Grandin 2002). As described above, concussive forces applied to the skull can induce unconsciousness with or without direct physical disruption of brain tissue. Inaccurate shot placement, head size, damp cartridges and poor equipment maintenance have been identified as reasons for poor stun quality in commercial slaughter settings (Grandin 2002). These factors also have the potential to affect the permanence of unconsciousness when animals are euthanased via captive bolt. The concern over returning sensibility has led to the recommendation that captive-bolt shot be followed by a secondary step to ensure death (Finnie 1997; American Association of Bovine Practitioners [AABP] 2013; AVMA 2013). Recommended secondary steps include exsanguination, pithing, or intravenous injection of concentrated salt solutions, such as potassium chloride. While relatively simple to apply to individual or even small numbers of animals, the application of a secondary step to ensure death increases the time required for euthanasia which could be problematic in the case of a large-scale disease outbreak or natural disaster. It could also increase the risk for the operator, particularly when animals must be euthanased in less-than-ideal situations, such as transportation accidents. Limb movement is common following captive-bolt shot and that movement could increase the risk for personnel attempting to administer intravenous injections. Exsanguination has the potential to create significant environmental contamination when an infectious disease may be involved. Internal exsanguination reduces the environmental contamination but may prevent effective necropsy examination. Pithing has the potential to increase the exposure of personnel to nervous system tissues. For these reasons, improving captive-bolt euthanasia techniques so that the need for a secondary step to ensure death could be eliminated would be ideal.

Another limitation of captive-bolt devices is that they have a limited depth of penetration. To ensure maximum penetration, the device must be held firmly against, and perpendicular to, the animal's forehead. The shot must also be precisely placed in order to maximise the opportunity to disrupt the brainstem. Traditional landmarks used for the placement of the captive-bolt shot are often ineffective for causing direct, physical disruption of the brainstem because the wound created by the bolt was often too far rostral to allow direct penetration of the brainstem (Gilliam *et al* 2012). A more recent study by the authors of this report identified an alternative set of landmarks for captive-bolt shot placement that increase the likelihood of disrupting the brainstem in adult cattle (Gilliam *et al* 2016). Differences in head shape, primarily due to breed and, to a lesser degree, age, change the position of the brain relative to external landmarks. The landmarks identified by Gilliam *et al* (2016) place the shot directly over the brainstem in all of the breed types included in the study. Placing the shot directly over the brainstem increases the ability to cause direct physical disruption of the brainstem as long as penetration depth is adequate.

The authors are aware of anecdotal reports that a non-penetrating captive bolt may be as or more effective than a penetrating bolt in neonatal calves. Non-penetrating captive bolts are used to stun cattle and other animals prior to slaughter but the authors are not aware of any published studies investigating their use for euthanasia.

The CASH Dispatch Kit (http://www.accles-shelvoke.com/usa-cash-dispatch-kit) is a pistol-style captive-bolt device designed specifically for on-farm euthanasia of a variety of livestock species. The system accommodates penetrating bolts of various lengths as well as a non-penetrating muzzle attachment, allowing the bolt type to be matched correctly with the type of animal being euthanased. The extended bolt provided with this kit is approximately 2.5-cm longer than the bolts of many traditional devices. The extended bolt length offered by the CASH equipment should provide increased opportunity to disrupt the brainstem in adult cattle, especially when combined with improved shot placement as described in the above referenced study. Five power loads of various strengths are also available to allow matching of the appropriate power load to the animal being euthanased.

Recognising the logistical issues of necessitating a secondary step, there is a need for a euthanasia method that has the availability, efficacy and safety of a captive bolt that does not require the application of a secondary step to ensure death. The purpose of this study was to evaluate the effectiveness of the CASH Dispatch Kit combined with alternative shot placement landmarks that maximise the opportunity to physically disrupt the brainstem as a single-step euthanasia method for cattle of various ages and breeds.

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Materials and methods

Study animals

Cattle requiring euthanasia for reasons unrelated to the study were identified. Information regarding age, breed, sex and reason for euthanasia were recorded. Animals were obtained through the veterinary teaching hospital at Oklahoma State University and co-operating farms. A small number of healthy cull animals were euthanased at a co-operating processing facility and carcases were processed once data related to the study had been collected. The cattle were grouped according to age into adult (> 24 months), young (6–24 months) and neonate (< 1 month) categories.

Captive-bolt equipment

A CASH Dispatch Kit was utilised for this study. The bolt length and power load combinations utilised for each group were based on the manufacturer's recommendations (see below for details).

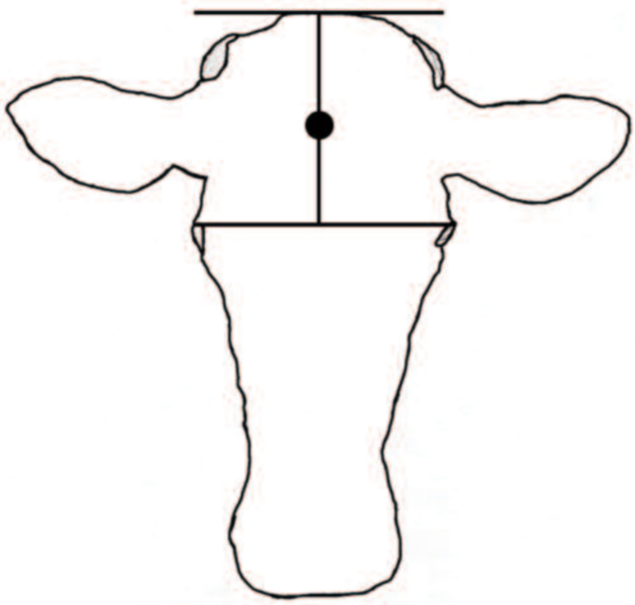
Euthanasia procedure

This study was carried out under field conditions and euthanasia performed in a variety of situations. For this reason, the individual euthanasia procedure for each animal varied to some degree. All cattle received a single shot from the CASH captive-bolt device. The bolt length and power load combinations were based on the animal's age and size and the manufacturer's recommendations. Generally, adult cattle were shot using the extended bolt and orange power load. Young cattle were shot with the medium (or standard) length bolt and blue power load. Neonatal animals were shot with the medium length bolt and yellow power load. An additional group of neonatal calves was shot with the CASH device fitted with a non-penetrating muzzle attachment and yellow power loads. In an effort to protect the welfare of each animal being euthanased, operators were afforded the flexibility to adjust the bolt length and power load upward should such adjustments be deemed necessary based on their own experiences. Mature bulls and a few very large cows were shot using the extended bolt and black power load. All cattle were euthanased by one of the authors (JG, JW or JH) or by a co-operating faculty member who had considerable experience with captive-bolt euthanasia.

The landmarks used for placement of the captive bolt were described in a previous report (Gilliam *et al* 2016) by the authors. Specifically, the shot was placed on mid-line at a point half-way between the top of the poll and a line drawn between the lateral canthus of each eye (Figure 1). This shot placement has been used in a recently published similar study (Derscheid *et al* 2016).

Restraint was provided by a variety of means depending on the particular situation. Recumbent animals were restrained via a halter tied to a hind limb. A few very gentle animals were restrained in a stall using a halter. Most ambulatory animals were sedated while restrained in a chute or head gate and allowed to become recumbent after being released. Sedation was provided using xylazine hydrochloride (Anased® injection, 100 mg ml^{-1} xylazine

Figure 1



Position of captive-bolt shot denoted as a point on midline half-way between the top of the poll and a line drawn between the lateral canthus of each eye.

(Lloyd Inc, Shenandoah, IL, USA) administered intramuscularly or intravenously at a dose of approximately 1 mg kg^{-1}. Most sedated animals became recumbent within 5–10 min. A few sedated animals remained standing but were unresponsive to the head being touched with the captive-bolt pistol. Sedation was used solely as a method of restraint to facilitate accurate shot placement without the need to have the animals restrained in a chute at the time of euthanasia. Cattle euthanased at the processing facility were restrained in a conventional concrete stunning box without head restraint or sedation.

Data collection

Immediately following the captive-bolt shot, the cattle were assessed for sensibility using the following criteria: immediate collapse (if standing), centered eye position and dilated pupil, lack of corneal reflex, lack of co-ordinated respiration, lack of vocalisation, and lack of a righting reflex (Gregory & Grandin 2007). Co-ordinated respiration was defined as rhythmic movement of the thoracic or abdominal wall with corresponding movement of air from the nostrils. Righting reflex was defined as co-ordinated attempts to regain sternal recumbency or rise. The presence of a heartbeat was determined via thoracic auscultation. In some calves, palpation of the chest wall was utilised as well. Signs of sensibility were monitored continuously and recorded at 1-min intervals. The presence or absence of a heartbeat was recorded at 1-min intervals. Once a heartbeat could no longer be detected, monitoring continued for an additional three minutes to ensure that the heartbeat did not return.

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Limb movement was assigned a kicking score of 0–3: (0) no movement; (3) repeated vigorous kicking at 1-min intervals. Time of last limb movement was recorded.

The protocol originally set 10 min as the maximum allowable time for an animal to persist with a heartbeat. The authors assumed that the heart would not beat for longer than 10 min in the absence of respiration. If the heartbeat continued at 10 min, it was stopped via intravenous injection of saturated potassium chloride (KCl) (approximately 340 g of KCL in a litre of water). After observing three animals that had no signs of sensibility but still had a heartbeat at 10 min, the protocol was amended to allow continued observation of the animal beyond 10 min as long as no signs of returning sensibility were present. This was done to allow more accurate investigation of the time to cardiac arrest when an animal is insensible and not breathing following captive-bolt shot.

If any signs of sensibility were noted, a secondary step was applied immediately to ensure the welfare of the animal. Any animal that failed to be rendered immediately insensible or exhibited a corneal reflex at any point received a second shot and was exsanguinated or KCL injection was performed. If any other sign of returning sensibility was noted during the observation period, KCL was immediately administered. For animals euthanased at the processing facility, an animal showing any evidence of returning sensibility received a second shot and was immediately exsanguinated.

For any animal showing evidence of returning sensibility, the head was collected post mortem and the bolt path determined by gross pathology assessment or by computed tomography.

Euthanasia was deemed successful if the animal was immediately rendered insensible and experienced cardiac arrest prior to any evidence of returning sensibility. If a secondary intervention was required for any reason, the euthanasia was deemed unsuccessful.

This study was approved by the Institutional Animal Care and Use Committee of Oklahoma State University.

Statistical analysis

Descriptive statistics were calculated using Excel® (Microsoft, Redmond, WA, USA). SPSS v 21 (IBM, Armonk, NY, USA) was used for analysis of between group comparisons. Comparison between groups for success/failure was done via contingency table and Chi-squared analysis. Comparisons between groups for time to cardiac arrest were done via Kruskal-Wallis, with a Bonferroni correction for multiple comparisons.

Results

A total of 74 animals were euthanased as part of this study. This included 34 adults, 19 young, and 21 neonatal animals. Of the adult and young animals, 46 were shot by JG, three were shot by JW, and four were shot by a co-operating faculty member. All of the neonatal animals were shot by JW or JH. The adult group included 30 cows and four bulls consisting of the following breeds: Holstein (14), Jersey (6), Angus (3), Polled Hereford (2), Beef cross (2), and Limousin, Brangus, Horned Hereford, Beefmaster, Simmental, Maine Anjou, and Belted Galloway (1 each). The young group included ten heifers, eight steers, and one bull consisting of the following breeds: Beef cross (8), Holstein (7), Jersey (3), and Limousin (1). The neonatal group included 21 bull calves. All of the calves were Holstein except for one Jersey/Angus cross. The calves were divided into two groups. One group was shot with a penetrating bolt (eleven calves) while the other was shot with a non-penetrating bolt (ten calves).

The most common reason for inclusion in the study was slaughter for salvage for the adult (5) and young (7) animals. These carcases were processed after data collection was complete. Severe dehydration and depression due to advanced scours was the most common reason for inclusion of neonatal calves (20). Lymphosarcoma and recumbency were other common reasons for inclusion of adult animals. All other animals represented a wide variety of infectious, metabolic, or traumatic conditions.

Three adult cows (two Jersey, one Holstein) were considered failures and excluded from further data analysis as a result of an intravenous KCl injection due to the persistence of a heartbeat at 10 min post captive-bolt shot. No signs of sensibility were observed in any of these animals prior to KCl injection. Based on these three animals, the protocol was amended to allow monitoring beyond 10 min as long as no signs of sensibility were present.

All animals in the study were rendered immediately insensible via a single shot. In the adult group, 90.3% (28/31) of the cattle were successfully euthanased via a single shot from the CASH penetrating captive bolt. Three cows (two Holstein, one Jersey) began to exhibit shallow, co-ordinated respiration at 1.5–5 min post shot. All other signs of sensibility were absent. One of these cows received immediate KCL injection. One cow was shot again because KCL was not immediately available. KCl was administered within 2 min of the second shot. Cardiac arrest occurred immediately after KCl injection in both cows. The third cow was shot again and immediately exsanguinated because the carcase was to be processed after data collection.

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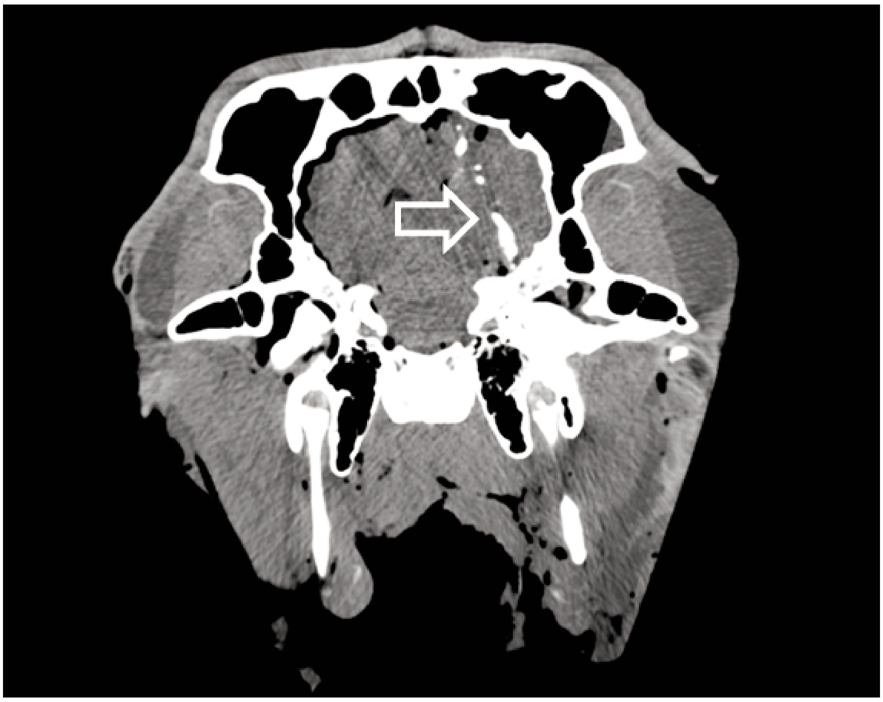
Figure 2



Transverse computed tomography image of the brain of an adult Holstein cow euthanased via penetrating captive bolt. Note that the bolt path (open arrow) angles into the right cerebral hemisphere missing the brainstem. This animal did not have a corneal reflex but began to exhibit shallow rhythmic respiration approximately 2 min post shot. A second shot was immediately applied followed by exsanguination.

In the young group, 89.5% (17/19) of the animals were successfully euthanased via a single shot from the CASH penetrating captive bolt. A crossbred beef heifer and a crossbred beef steer began to exhibit shallow co-ordinated respiration at 8 and 9 min post shot, respectively. No other signs of sensibility were present and both animals immediately received KCl injection.

None of the neonatal calves exhibited any evidence of returning sensibility throughout the observation period although ethical and practical concerns led to the application of a secondary step in three calves (two calves and one calf in the penetrating and non-penetrating bolt groups, respectively). In the neonatal calf/penetrating bolt group, 81.8% (9/11) of the calves were successfully euthanased via a single shot from the CASH penetrating captive bolt. The heartbeat in two calves persisted beyond what was considered a reasonable time (20 min in these cases). One calf received a second shot at 26 min and cardiac arrest occurred at 30 min. The other calf received a second shot at 20 min and still had a very faint heartbeat at 60 min. The calf was exsanguinated at that time.

In the neonatal calf/non-penetrating bolt group, 90% (9/10) of the calves were successfully euthanased via a single shot from the CASH captive bolt with non-penetrating muzzle attachment. In one calf, the heartbeat was still present at 25 min. The calf was shot with a penetrating bolt at that point and cardiac arrest occurred at 28 min following the initial shot. Examination of the brains of the neonatal calves from both groups was not possible due to the location at which the euthanasia was performed.

The brains of all adult and young animals that required a secondary step and the brains of a few selected successfully euthanased animals were examined by necropsy or computed tomography. For each animal that resumed respiration, examination of the brain revealed that the bolt failed to directly penetrate the brainstem. The reasons for missing the brainstem consisted of slightly inaccurate shot placement or slight deviations of shot angle (Figure 2).

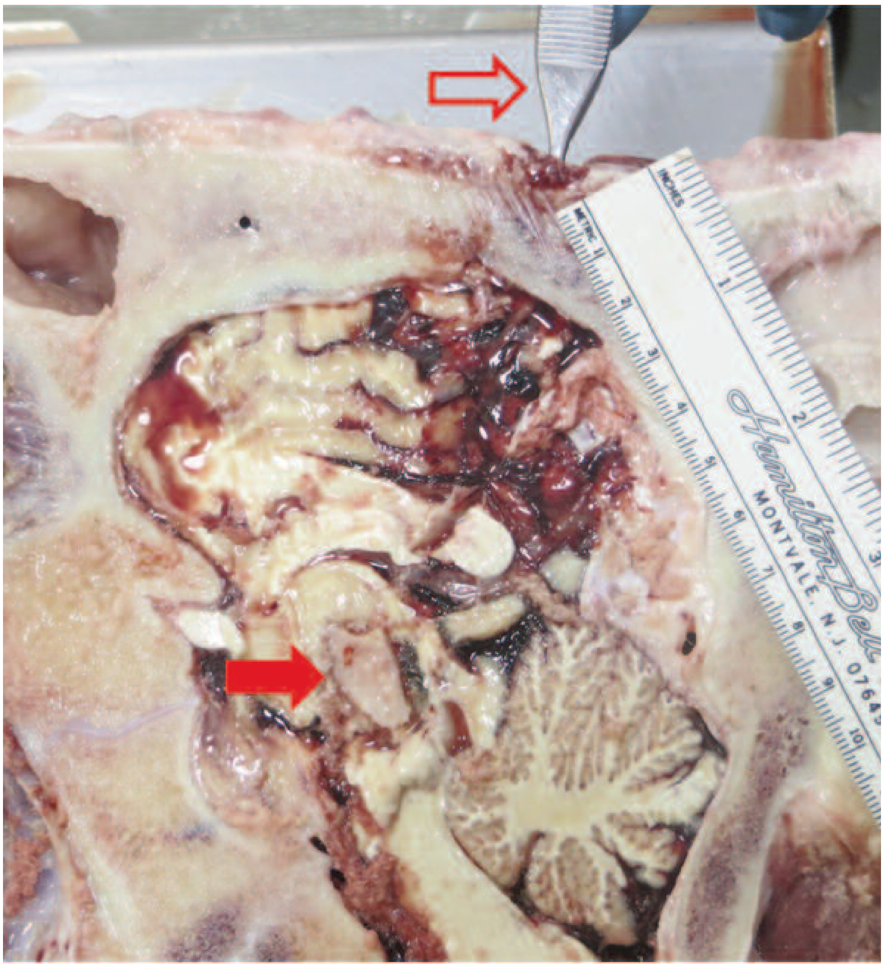
In the successfully euthanased cases that were examined post mortem, the captive-bolt shot resulted in direct physical disruption of the brainstem by the bolt or bone fragments in all but one of these cases (Figure 3). Of particular interest was a mature Horned Hereford bull. Following application of the captive bolt, no signs of sensibility were present and cardiac arrest occurred in three minutes. At necropsy, it was determined that the bolt penetrated into the third ventricle but did not physically penetrate the brainstem due to inadequate depth of penetration.

There were no differences (P = 0.889) in the proportion of animals euthanased successfully between the different age classifications or bolt types (neonatal calves).

A total of 12 animals (nine adult, three young) received sedation prior to euthanasia. There were no differences (P = 1.00) in euthanasia success/failure in animals that received sedation versus those that did not.

For all animals that were successfully euthanased via a single shot, the time to cardiac arrest and time of last observed movement varied widely (Table 1). There were no differences in time to cardiac arrest or time to last movement between any of the groups (P = 0.19). Across all age groups, the presence of a heartbeat was detected beyond ten minutes in multiple animals.

Figure 3



Necropsy photo of the brain of an 18-month old Limousin bull euthanased via penetrating captive bolt. The entry point of the bolt is indicated by the metallic probe (open arrow). Note the bone fragment pushed deep into the brainstem (solid arrow). Cardiac arrest occurred 8.5 min post shot.

Discussion

Based on the findings of this study, a single shot from a CASH captive bolt with appropriately selected bolt length and power load can normally euthanase cattle of various breeds and ages when the shot is placed half-way between the poll and a line drawn between the lateral canthus of each eye. Overall, 88.7% of the animals euthanased in this study were successfully and humanely euthanased via a single shot from the CASH device. It is important to note that all of the animals in the study were rendered immediately insensible based on established criteria for assessing stun quality in slaughter facilities (Gregory & Grandin 2007).

In all animals that exhibited evidence of returning sensibility, the only sign observed was a resumption of co-ordinated respiration. The return to rhythmic breathing has been described as one of the earliest indicators of returning sensibility in pigs following electrical stunning (Anil 1991). No animal exhibited a corneal reflex, vocalisation, or a righting reflex at any point during the observation period. It is unknown whether other signs of sensibility would have eventually returned in these animals. The animals were monitored very closely and a secondary step was applied immediately when respiration was noticed.

Post mortem examination revealed that the captive-bolt shot failed to physically disrupt the brainstem in each of these cases. Failure to penetrate the brainstem occurred for a variety of reasons. Holding the captive-bolt device at a slight medial-to-lateral angle rather than perpendicular to the skull resulted in the bolt entering the skull at the intended location yet passing into the cerebral hemisphere and missing the brainstem in two animals. One of these animals was in a stunning box without head restraints and the animal moved slightly as the shot was fired causing the bolt to enter the head at an incorrect angle. The other animal was in an awkward position prior to euthanasia and the operator inadvertently held the gun at a slight angle. A prominent bony ridge on the head of another cow caused the gun to slip laterally as it was fired. In that cow, the bolt penetrated completely through the cerebral cortex but was lateral to the brainstem. A heifer that was standing at the time of euthanasia moved slightly as the shot was fired causing the bolt to enter the skull more rostrally than intended. The rostral position caused the bolt to penetrate the rostral aspect of the cerebral hemispheres but missed the brainstem. A steer was lying in lateral recumbency at the time of euthanasia. Long hair at the top of the poll caused the operator to misjudge the position of the poll resulting in

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the shot being placed too high on the head. The bolt entered the caudal aspect of the cerebral hemisphere and cerebellum but did not penetrate the brainstem. These findings highlight the importance of adequate restraint along with careful shot placement and technique to maximise the effectiveness of the captive-bolt device. Slight movement of the animal, minor errors in shot placement, or holding the gun at a slight angle can all result in the bolt failing to penetrate the brainstem. Failing to physically penetrate the brainstem may result in a resumption of co-ordinated respiration several minutes after a captive-bolt shot even if other signs of sensibility are absent. Inaccurate shot placement has been documented as a cause of poor stunning in slaughter facilities (Grandin 2002; Atkinson *et al* 2013).

The brains of all successfully euthanased animals were not examined. However, a few selected cases were examined at necropsy. Of particular interest was a mature bull in which the bolt penetrated the third ventricle but failed to penetrate deep enough to directly disrupt the brainstem. This case provides evidence that a penetrating captive bolt can be effective as a single-step euthanasia method even if the physical penetration of the brainstem does not occur. It is likely that the brainstem in this animal was disrupted by shockwaves that reached deeper than the physical limitations of the bolt since the bolt path ended in the third ventricle directly above the brainstem (Lambooy 1982). Even though euthanasia was successful in this particular case, direct physical disruption of the brainstem is preferred.

A few challenges to correct shot placement were discovered during the study. These challenges were most notable in Jersey cows. Several of the Jersey cows included in the study had a prominent bony ridge at the symphysis between the right and left frontal bones. The shot-placement landmarks used for this study resulted in the captive-bolt device being placed directly on that bony ridge. When the device was fired, it was difficult to keep it from slipping off to one side of the ridge. This problem occurred in two of the cows that were euthanased early in the study but subsequently excluded due to the persistence of cardiac function beyond ten minutes and in one cow that received KCL due to resumption of respiration. The brainstem was missed in all three of those cows due to the lateral position of the bolt path relative to the brainstem. For subsequent cows that had a similar bony ridge, the authors placed the captive bolt just lateral to the ridge and at a slight angle so that the bolt path was directed back toward the brainstem. This adjustment only had to be made for a few cows but appeared to be effective. A second issue was the concave shape of the forehead of some Jerseys. In one Jersey heifer, the bolt entered the skull at the desired location but placing the captive bolt perpendicular to the frontal bone resulted in a bolt path that penetrated the brain at a more caudal angle than desired. Even though the angle was not perfect, this particular animal was euthanased successfully. Further research is needed to further define the impact of Jersey head shape on captive-bolt placement.

Table 1 Median (range) time to cardiac arrest and last observed movement in cattle of various ages euthanased with the CASH Dispatch Kit captive-bolt device.

	Time (min post captive-bolt shot), median (range)	
Group	**Cardiac arrest**	**Last movement**
Adult	7.0 (1.0–16.0)	3.0 (0.0–10.0)
Young	8.33 (2.0–13.0)	4.5 (0.0–8.0)
Neonate-penetrating	5.0 (0.0–25.0)	3.0 (0.0–24.0)
Neonate-non-penetrating	5.0 (0.0–16.0)	0.0 (0.0–8.0)

Detection of an isoelectric electroencephalogram (EEG) is the ideal method for confirming brain death. Application of an EEG was not possible in this study due to many of the animals being euthanased under field conditions. The loss of brainstem reflexes and cessation of cardiac function were used as indicators that death had occurred. At the beginning of the study, ten minutes was chosen for the cut-off at which a secondary measure would be applied if the heartbeat was still present. This time-point was chosen because it was assumed that the heart would not function for longer than ten minutes in the absence of respiration. It was quickly discovered that the heart can function beyond the ten-minute time-frame even when all signs of sensibility are absent, so the protocol was amended to allow monitoring for a longer period as long as no signs of sensibility returned. Three animals were eliminated from further inclusion in the study due to this change. In one of these three cows, the bolt directly penetrated the brainstem. In the other two, the bolt path was slightly more lateral than intended, resulting in failure to directly penetrate the brainstem.

The persistence of cardiac function for several minutes after the captive-bolt shot in animals that are adequately stunned and show no signs of sensibility is concerning due to the importance of cardiac arrest to confirm death in field settings. Several authors have documented cardiac function for variable periods of time after brain death in swine (Turner *et al* 2012; Woods 2012; Casey-Trott *et al* 2013, 2014) and poultry (Turner *et al* 2012). Prior to the current study, the authors are unaware of similar data for cattle.

The persistence of cardiac function in the absence of respiration appeared to be even more variable in neonatal calves compared to older groups. Persistence of cardiac function led to the application of a secondary step in three neonatal calves even though no signs of sensibility were evident. Cardiac function persisted beyond 20 min after captive-bolt shot in these calves. Waiting longer for cardiac function to cease was considered impractical so a secondary step was applied in these animals. In the most extreme case, one calf still had a faint heartbeat at 60 min after having been shot twice with a captive-bolt device. No evidence of respiration was present in that calf. Although persistence of cardiac function appeared to be more variable in neonates, no significant differences between age groups were observed.

In similar studies investigating euthanasia of swine via non-penetrating captive bolt, Casey-Trott *et al* found that cardiac function persisted longer in neonatal pigs (< 3 days) (Casey-Trott *et al* 2013) compared to pigs weighing 3–9 kg (Casey-Trott *et al* 2014). It has been reported that glycogen stores within the heart of human neonates can enable cardiac function to persist up to 20 min in an anaerobic environment (Aroni *et al* 2012). It is possible that a similar situation may occur in neonatal calves and that could explain the persistence of cardiac function for extended periods in some calves. It has also been reported that brains of neonatal piglets are more resistant to cortical impact (Duhaime *et al* 2000) and subdural haematoma (Durham & Duhaime 2007) when compared to older pigs. It is possible that the brain of neonatal calves is more resistant to the effects of the captive bolt compared to the brains of older animals.

As discussed above, accurate shot placement is required for successful captive-bolt euthanasia. Adequate restraint to minimise movement is a key component of accurate shot placement. A chute and/or headgate combined with the use of a halter will adequately restrain most ambulatory animals. Outside of a research setting, physical restraint for captive-bolt euthanasia is generally adequate and sedation is not required. However, due to the study design, euthanasing animals restrained in a chute was not possible because the investigators needed ready access to the animal to monitor signs of sensibility and cardiac function and the limbs needed to be free in order to evaluate limb movement following the captive-bolt shot. For this reason, most ambulatory animals were sedated, released from restraint and allowed to become recumbent and/or sedate prior to euthanasia. If ambulatory animals were not sedated, physical restraint, such as a chute or headgate, would have been required. The animal described above which moved at the time of the shot is a good example of how inadequate restraint can affect shot placement and euthanasia outcome. That particular calf was blind and very calm. She was not sedated and restrained only by a halter being held by the investigator operating the captive bolt. Slight movement at the time of the shot resulted in inaccurate shot placement. Had she been restrained in a chute with the head secured with a halter or been sedated, this movement would most likely have not occurred. Additional benefits of sedation include reduced stress for nervous or fractious animals and easier carcase removal when carcases do not have to be removed from a restraint device such as a chute.

The duration and severity of limb movement following captive-bolt shot was highly variable. In most cases, limb movement ceased a few minutes prior to cardiac arrest. This finding indicates that cessation of limb movement should not be used as an indicator of death because cardiac function often persists beyond the last observed limb movement. In some animals, there appeared to be a correlation between the duration of cardiac function and the duration and intensity of limb movements. Many of the animals that exhibited persistent cardiac function also exhibited persistent, and sometimes more vigorous, limb movements. In some animals, the observer noted the sound of air movement from the nose or mouth that coincided with vigorous kicking of the hind limbs. Careful observation failed to reveal co-ordinated respiration and the observer felt that the limb movement was possibly creating enough air movement in the respiratory system to maintain cardiac function for a longer period. However, in several other animals, cardiac function persisted longer than expected in the absence of vigorous limb movements.

This study is unique in that it is one of very few to have investigated the use of a captive-bolt device specifically for euthanasia when a secondary step is not applied immediately after stunning.

Animal welfare implications and conclusion

A single shot from the CASH Dispatch Kit captive-bolt system combined with improved shot placement landmarks effectively stunned all of the animals in this study and humanely euthanased 88% of the animals without the need for a secondary follow-up step. This high rate of success was dependent on accurate shot placement which can only consistently be achieved with good animal restraint. In some cases, sedation may be the most appropriate form of restraint and some of the animals in this study were sedated prior to euthanasia for the purpose of restraint. Animals in the study ranged from neonatal calves to mature bulls indicating the ability to effectively euthanase a wide range of cattle. The goal of this study was to determine if the CASH captive-bolt equipment and improved shot placement landmarks would perform well enough to eliminate the need for a secondary step to ensure death. Due to the potential welfare implications of the topic, eliminating the secondary step would require that virtually 100% of the animals be effectively euthanased without a follow-up step. Unfortunately, approximately 12% of the animals in this study exhibited signs of brain activity. Further investigation revealed that the brainstem was missed in each one of these animals due to slight imperfections in shot placement or angle. These findings reiterate the need for precise shot placement and execution when cattle are euthanased via penetrating captive bolt. While the CASH equipment was used in this study, it is likely that other captive-bolt devices with power and penetration depth adequate to reach the brainstem would have similar effectiveness when combined with the shot-placement landmarks used in this study. An additional concern identified in this study was that cardiac function persisted for several minutes following application of the captive bolt even when no signs of sensibility were present. An animal should never be considered dead if cardiac function remains. Limb movement typically stops before cardiac arrest and, therefore, should not be used to determine death. Rapid induction of unconsciousness is important for ensuring the welfare of animals during euthanasia. That goal was accomplished in this study. In the majority of animals that unconsciousness was permanent. Since a small percentage of animals in the study exhibited brain activity after application of the captive bolt, the authors recommend monitoring for signs of sensibility until cessation of cardiac function or application of a secondary step to ensure death when a captive bolt is used for euthanasia of cattle. Further work is needed to develop equipment or techniques that will eliminate the need for a secondary follow-up step to ensure death during captive-bolt euthanasia.

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