Evaluation of spring-powered captive bolt guns for dispatch of kangaroo in-pouch T. M. Sharp^{1,2*}, S.R. McLeod³, K.E.A. Leggett¹ and T.J. Gibson⁴ ¹Current address: Fowlers Gap Arid Zone Research Station, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW 2052 Australia. ²School of Biological Sciences, University of Wollongong, NSW 2522 Australia. ³Vertebrate Pest Research Unit, Forest Road, NSW Department of Primary Industries, Orange NSW 2800 Australia. ⁴Department of Production and Population Health, Royal Veterinary College, University of London, Hatfield, AL9 7TA, United Kingdom *Corresponding author, Telephone: +61 401 285 913 Email: trudy@awscience.com

31 Abstract

Context. During commercial harvesting or non-commercial kangaroo culling 32 programs, furred pouch young of shot females are required to be euthanased to 33 34 prevent suffering and because they would be unlikely to survive independently. 35 However, the current method (a single, forceful blow to the base of the skull) is applied inconsistently by operators and perceived by the public to be inhumane. 36 37 Aims. To determine if an alternative method for dispatching pouch young— a springoperated captive bolt gun—is practical and effective at causing immediate 38 39 insensibility in kangaroo pouch young. *Methods.* Trials of the spring-operated captive bolt guns were conducted first on the 40 heads of pouch young cadavers and then on live pouch young, during commercial 41 harvesting. Performance characteristic of the spring-operated guns were also 42 43 measured and compared with cartridge-powered devices. **Key results.** The captive bolt guns caused insensibility in only 13 out of 21 trials on 44 live pouch young. This 62% success rate is significantly below the 95% minimum 45 acceptable threshold for captive bolt devices in domestic animal abattoirs. Failure to 46 stun was related to bolt placement, but other factors such as bolt velocity, bolt 47 48 diameter and skull properties such as density might have also contributed. Springoperated captive bolt guns delivered 20 times less kinetic energy when compared with 49 cartridge-powered devices. 50 *Conclusions.* Spring-operated captive bolt guns cannot be recommended as an 51 acceptable or humane method for dispatching kangaroo pouch young. 52 53 *Implications.* Captive bolts guns have potential as a practical alternative to blunt head trauma that may standardise dispatch technique and reduce animal (and observer) 54 distress. However, operators must continue to use the existing prescribed dispatch 55 56 methods until cartridge-powered captive bolt guns have been trialled as an alternative bolt propelling method. 57 58 59 Keywords: kangaroo harvesting, captive bolt gun, culling, euthanasia, blunt trauma, animal welfare, humaneness, joey 60

Introduction

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In Australia, all states and territories have legislation to protect kangaroos, however, under strict government control, four of the most abundant species are harvested commercially (by shooting) for meat and skin products. Kangaroos are also shot during non-commercial culling to reduce population size and thereby reduce negative impacts on the environment or agricultural production. Commercial and non-commercial shooting differ in that commercial shooters must be licensed and require a higher level of training compared with noncommercial shooters. Also, commercial harvesting must be done in accordance with a government approved management plan and compliance with a code of practice (Anon 2008a) is monitored. Minimum animal welfare standards for both commercial and non-commercial shooting of kangaroos are prescribed in national codes of practice (Anon 2008a; Anon 2008b). Both codes require that dependent young of shot females must be euthanased to prevent them from suffering. Specified acceptable euthanasia methods for small, furless pouch young (i.e. that fit within the palm of the hand) are either a 'single forceful blow to the base of the skull sufficient to destroy the functional capacity of the brain' or 'stunning, immediately followed by decapitation by rapidly severing the head from the body with a sharp blade'. Furred pouch young must be dispatched by a 'single forceful blow to the base of the skull sufficient to destroy the functional capacity of the brain'. Although the codes of practice do not provide specific guidelines on how to apply the single forceful blow to the head, commercial kangaroo shooters usually do this by holding the joey by the hindquarters and swinging it in an arc so that its head hits a hard object such as a large rock or side of the rack or tray on their vehicle. Larger furless joeys are sometimes placed onto the ground and the head is stomped on with the foot and occasionally shooters use a heavy bar or pipe to hit the joey on the head whilst holding them by the back-legs (McLeod and Sharp in press). All of these procedures fit within the codes' loose definition of a 'single forceful blow to the head' as described

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According to international guidelines on euthanasia, manually applied blunt trauma to the head can be a rapid and humane method of dispatching small animals such as birds, amphibians, fish, reptiles and some neonatal animals with thin skulls (e.g. pigs) (AVMA Panel on Euthanasia 2013). However, to be effective and humane, the method must be applied using a single sharp blow delivered to the cranium with sufficient force to produce immediate depression of CNS (central nervous system) function and destruction of brain tissue, producing irrecoverable concussion leading to death. Although, considered a humane method of dispatch when performed correctly, this technique is often seen as undesirable as it is unsightly and emotionally unpleasant for both observers and operators. There is also a reluctance of some operators to perform dispatch by blunt force trauma. When dispatching joeys, if the operator does not deliver the blow with sufficient force or does not contact the correct position on the head, then there is the potential that the animal will not be rendered completely insensible and it could experience pain and distress. Some guidelines consider blunt trauma to be only acceptable in instances where it is the most rapid and practical method available (e.g. for the emergency euthanasia of injured newborn piglets, CCAC 2010). Experts on euthanasia have also recommended that blunt trauma should be replaced when possible with alternative methods (AVMA Panel on Euthanasia 2013). However, some of the alternatives suggested are not suitable for use on wild animals in field situations. For example, it has been proposed that, during harvesting, joeys should be euthanased with a lethal injection administered by a veterinarian (NSW Young Lawyers Animal Law Committee 2008 cited in Boom and Ben-Ami 2011). This would involve distress and pain associated with handling, restraint and the injection. Also, it would be impractical and expensive to carry out and there would be negative consequences for non-target animals that scavenge carcasses that are not disposed of correctly. The methods currently used to dispatch kangaroo joeys generate considerable controversy.

Blunt trauma to the head is perceived to be inhumane, cruel and violent by a number of

animal and kangaroo protection groups (e.g. Animal Liberation undated; Australian Wildlife Protection Council undated; Gellatley 2009; Wilson 2005). Likewise, the media are prone to describing culling methods using emotionally charged language, for example, 'Orphaned joeys face a bloody and barbaric death' (Holland 2009). A recent survey showed that the Australian public have strongly negative attitudes towards blunt trauma as a dispatch method (McLeod & Sharp in press). Furthermore, the Royal Society for the Prevention of Cruelty to Animals (RSPCA) has also questioned the appropriateness of the techniques prescribed for dispatching pouch young and proposed that research should be urgently conducted to determine what methods are the most humane (RSPCA Australia 2002; 2009b). A potential alternative to blunt trauma would be the dispatch of joeys with captive bolt guns. Captive bolt guns fire a steel bolt that either penetrates (penetrating captive bolt) or impacts (non-penetrating captive bolt) the cranium transferring the kinetic energy of the bolt to the head and brain. The aim is to cause concussion and damage (focal and diffuse) to the CNS, resulting in rapid insensibility (Gregory 2007). These weapons are powered with blank gunpowder cartridges, compressed air or a spring mechanism. Stunning with a captive bolt gun is typically followed up immediately with a secondary killing method, while the animal is still unconscious, to ensure a prompt death without recovery. For example, when cattle are slaughtered for human consumption, they are often stunned with a captive bolt gun and then exsanguinated. However, it has also been reported that captive bolt devices can be used as a single-step method for killing cattle (Gilliam et al. 2012) and sheep (Gibson et al. 2012) without the need for sticking or pithing, when shot in the correct position. Although, mostly used for the stunning of larger animals (sheep and cattle), captive bolt guns have also been developed for use on smaller animals including poultry (Raj & O'Callaghan 2001), dogs (Dennis et al. 1988) and rabbits (Holtzmann 1991). The recommended stunning positions vary widely between species, principally due to differences in the anatomy of the head and skull. In rabbits, the currently recommended stunning position is on the top of the head at the midline between the base of the ears

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(Holtzmann 1991; EFSA AHAW panel 2006). There have been no studies on the use of captive bolt guns for the stunning or the killing of kangaroo pouch young. When blunt trauma is applied to the head, young are usually first removed from the pouch. This removal and subsequent handling can cause struggling and vocalising, likely to be indicators of fear and distress (McLeod & Sharp, 2014). Applying the captive bolt to the head of the joey whilst it remains within the pouch could potentially minimise the distress associated with handling. Spring-powered captive bolt devices, which are used to stun small animals such as rabbits and poultry, are compact and portable, and so would be convenient for using in field situations. They are also lighter than and relatively inexpensive compared with the blank gunpowder cartridge-powered devices commonly used on larger animals, and do not require a licence 624 Wildlife Research T. M. Sharp et al. to own or operate, as is the case in some states in Australia, Informal discussions with harvesters and a representative of the NSW Kangaroo Management Agency (NSW Office of Environment and Heritage, Kangaroo Management Section) before the present study indicated a preference for testing the spring-powered devices because of these advantages. Thus, the aim of the study was to determine whether commercially available spring-powered penetrative captive bolt guns are effective for the killing of pouch young during commercial harvesting or non-commercial culling of kangaroos.

Materials and Methods

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The project was conducted in accordance with the Australian code of practice for the care and use of animals for scientific purposes (NHMRC 2004) with approval of the NSW Department of Primary Industries Animal Ethics Committee (Animal Research Authority number ORA 10/012).

Initially, we tested two different models of spring-powered penetrating captive bolt guns on the heads of carcasses. These were the Dick KTBG (Friedr. Dick GmbH and Co, Deizisau, Germany) and the Finito (Klaus-Gritsteinwerk GmbH and Co, Bünde, Germany) (see Fig. 1).

Both types of captive bolt guns were compact, lightweight and easy to disassemble for cleaning; also, when fired into the skull, they appeared to cause wound tracts of similar depth and trajectory. However, with the Dick KTBG it was much easier and quicker to engage the spring and also to fire the bolt. Thus, for all the subsequent tests on carcass heads and live animals, the Dick KTBG captive bolt gun was used.

With the tests in dead animals, we assessed the degree of skull and brain damage caused by the bolt and also examined skull properties such as thickness. This information was then used to determine potential captive bolt placement sites, with the aim of causing extensive damage to specific brain structures (cerebral cortex and brainstem). We then assessed the effectiveness of the spring-powered captive bolt in causing irrecoverable insensibility in live animals. An accepted welfare standard in livestock abattoirs is that the first shot must instantly induce insensibility in 95% of animals (Grandin 2010) and this standard was adopted as a threshold for effectiveness in the study. The performance characteristics (bolt velocity, kinetic energy, penetration depth) of spring-powered captive bolt guns were also examined in the laboratory. All kangaroo pouch young used in the tests on live animals were to be killed during commercial harvesting and were not selected separately for the study.

Trials on cadaver heads

In total, 15 heads from dead eastern grey kangaroo (Macropus giganteus) young were used to examine the penetration characteristics of the captive bolt guns and macroscopic damage to skull and brain structures. Ten of the carcasses were sourced from veterinary clinics, and five were obtained from commercial kangaroo shooters. The origin of every animal was not known; however, most of those from the veterinary clinics had been euthanased with an injection of barbiturate because of sickness or injury and some had been found dead as a result of trauma from a collision with a motor vehicle. The animals from the shooters had either been found dead or killed using decapitation. On the basis of head measurements, the

age of the young ranged from 105 to 306 days (Poole et al. 1984). The heads were frozen for storage at -20C and defrosted for 18–24 h before testing.

One operator performed all of the trials on the cadaver heads. Each head was shot once on the highest point of the head at the midline, with the gun held at a perpendicular angle to the skull. After firing, the skulls were skinned and the position, shape and size of the bolt entrance cavity on the cranium recorded. Trajectory and penetration depth of the bolt was measured from the outer surface of the skulls using a wooden probe inserted through the bolt entrance cavity. The heads were sawed (with a hacksaw) longitudinally through or near to the bolt penetration site. The skull, brain and specific brain structures were visually assessed. Skull thickness at various points was measured and damage to the brain was recorded using digital photographs. Skull thickness and bolt penetration depth were measured using digital vernier calipers (JBS tools).

Trials on live animals

The Dick KTBG captive bolt was used on a total of 21 live animals (eight red kangaroos (Macropus rufus), one western grey (Macropus fuliginosus) and 12 eastern grey kangaroos) to determine the effectiveness at causing insensibility. The animals were partially furred to fully furred, pouch young, with bodyweights ranging from 0.5 to 3 kg and all were >15 cm from head to the base of the tail. Pouch young age was determined on the basis of previous studies that examined the relationship between known-age and head (or tail) length (Sharman et al. 1964; Poole et al. 1982, 1984).

Two operators trained in the use of the captive bolts gun performed all testing on live animals. Immediately after a female kangaroo was shot, the carcass was located and the captive bolt was tested on the pouch young that were of a suitable size (approximately >15 cm from head to base of tail). The shots were aimed on midline at the highest point on the head with the gun perpendicular (i.e. at an angle of 90 degrees) to the skull. Two different methods of applying the bolt were used. Three pouch young were shot through the skin of the pouch, with the

orientation of the head determined by direct palpation. The muzzle of the captive bolt gun was placed firmly against the pouch skin and aimed for the crown of the head. However, with this approach, it was difficult to accurately locate the top of the head through the pouch; therefore, this method was used only a limited number of times. With all of the remaining young, the head only was uncovered from the pouch, and the captive bolt was applied directly to the crown. Immediately after shooting, all animals were examined for clinical signs of insensibility including sudden loss of muscle tone (body going limp), lack of purposeful or coordinated movements (such as raising the head), absence of corneal and palpebral reflexes, absence of pain response to toe pinch and absence of vocalisation. The presence or absence of normal rhythmic breathing and a heartbeat were also noted for each animal. Instantaneous insensibility after one shot was scored as a successful (or effective) shot, while any sign of sensibility was scored as unsuccessful (or ineffective) shot. Animals effectively stunned were observed for 5 min and time to recovery or death was noted. Animals not effectively stunned were immediately re-shot or euthanased. When euthanasia was performed, it was done by blunt trauma to the head, decapitation or IV overdose of barbiturate. The heads of 17 pouch youngs were collected and frozen for future examination. Six of the heads were thawed at room temperature and examined with computed tomography (CT). These heads were then frozen and thawed again prior to dissection. All heads were examined macroscopically as described for the dead-animal tests. Where possible, severity of damage to specific areas of the brain was examined from photographs of sagittal sections. Damage was assessed subjectively and graded as none, mild, moderate or severe. Damage to the left and right lobes of the cerebrum were grouped to aid analysis. Performance of spring-powered captive bolt guns

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The velocity of the spring-powered captive bolt guns (two Dick KTBG guns and one Finito

gun) was measured with a custom-built velocity meter (Solutions for Research Ltd, Silsoe,

Bedford, UK). The meter measured velocity of the bolt as it transects a series of seven infrared light-emitting diodes (LED). Each LED is positioned 4 mm apart and the time taken to transect consecutive LEDs was used to calculate the bolt velocity. Spring-powered captive bolt guns were fired 40 times for velocity assessment using the meter. Peak velocity was taken as the highest mean velocity recorded. The weight of each captive bolt, minus the spring was measured (10 replicates) on a precision balance (Acculab Vicon VIC-123, Acculab UK, Sartorius Group, Epson, Surrey, UK). Peak velocity of the bolt was recorded and used to calculate the kinetic energy of the bolt (*Kinetic energy* = $(\frac{1}{2} \times m) \times v^2$; where m = mass of the bolt (kg) and $v = \text{peak velocity (m.s}^{-1})$). By determining the kinetic energy, the two different captive bolt gun models were compared whilst taking into account differences in bolt weight. Peak velocity of the spring-powered captive bolt guns was compared with those generated by the cartridge powered .22 Cash Special (Accles & Shelvoke, Sutton Coldfield, UK) with 110 (clear 1.0 grain (gr)) and 170 (pink 1.25 gr) mg nominal powerloads (Gibson et al. submitted). Penetration depth was measured with the firing of the captive bolt guns into ballistics gelatine moulds. Five shots were fired 30 mm apart with the mean penetration depth calculated. The ballistics gelatine was prepared according to Fackler and Malinowski (1988). The diameter and length of the Dick KTBG bolt was 4.7 and 30 mm respectively, while for the Finito it was 5.4 and 33 mm respectively.

Statistical analysis

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Statistical analyses were done using the R language, version 3.0.3 (R Core Team 2014) and contributed packages. The R package 'nlme', version 3.1-117 (Pinheiro *et al.* 2014) was used to fit a mixed effects model that compared the peak velocity of the bolt from cartridge fired captive bolt guns (Cash Special) using 1.0 and 1.25 gr loads, with the peak velocity of the spring powered captive bolt devices (Dick KTBG and Finito). In the fitted model, type of captive bolt gun (cartridge or spring powered) was the fixed effect and each device was included as a random effect.

The R package 'Barnard', version 1.3 (Erguler 2012), was used to perform Barnard's unconditional test of the equality of two binomial probabilities. The test compared the equality of the probability of an effective shot on whether the bolt was fired into the brain from a position either at the crown/in front of the crown (rostral), or behind the crown (caudal). We also examined the effect of the independent variables, namely species, age, skull thickness and boltpenetration depth, on the likelihood that the captive bolt would render a pouch young insensible. We first used the R function 'glm' (R Core Team 2014) to fit full and nested generalised linear models to these data, specifying a binomial error distribution. The significance of the independent variables was determined by comparing the full and nested models with the restricted model, by using the likelihood-ratio test. In addition, the relationship between insensibility and damage to specific brain areas was also examined using logistic regression.

Results

Trials on cadaver heads

The Dick KTBG captive bolt was used on the heads of 15 eastern grey kangaroo cadavers.

The mean age of these animals was 183 days (\pm 61 SD). The most appropriate captive bolt

shooting position was determined to be at the highest point of the head on the midline (i.e. the

crown) where the skull was thin (1 mm thick) and the bolt would cause trauma to the

cerebrum and brainstem.

Mean skull thickness at the captive bolt entrance cavity was $1.00~(\pm~0.32~\mathrm{SD})$ mm and the mean bolt penetration depth was $27~(\pm~3.5~\mathrm{SD})$ mm. The captive bolt gun consistently produced a large entrance cavity (7-8mm in diameter) in the skull, which was approximately twice the diameter of the bolt. The bolt produced a well-defined wound tract, which extended into the cerebrum, almost extending the full thickness of the brain including the brainstem.

However, this tract was difficult to determine in some heads due to freezing and thawing

disrupting the fine details of structure in the brain. Fragments of bone and skin were also pushed into the wound tract with some heads.

When shooting in the crown position, we observed some cases of skin slippage', the movement of the skin across the underlying skull (Gregory 2007, pp. 196). This resulted in the bolt being misplaced, to the right or left of the midline and/or to the front (rostral) or to the back (caudal) of the crown. If skin slippage occurs during shooting of live animals, it could cause the captive bolt to enter the brain at the incorrect position, potentially resulting in incomplete concussion. To minimise the risk of skin slippage, the muzzle of the captive bolt gun should be placed flat (without angling of the gun) on the surface of the head. Also, excessive pressure should not be exerted on the head because this can result in slippage of the gun before and during discharge.

Trials on live animals

Pouch young showed variable responses to captive bolt shooting (Table 1). Animals that were effectively rendered insensible, most commonly went limp with the eyes closed. They also failed to respond to toe pinch nor did they vocalise or have corneal and palpebral reflexes. The most common indicators of incomplete concussion were eye blinking, a positive corneal reflex, vocalisations and coordinated movements. In some of the animals that were not rendered undoubtedly insensible some indicators of altered consciousness were observed, for example deep pain reflexes were lost despite corneal reflexes being present.

Of the 13 animals that were rendered immediately insensible after an initial shot, four regained sensibility after approximately 1 min and were subsequently euthanased. Animals that remained insensible after a minute either died or were euthanased without regaining sensibility.

Of the eight pouch young that *were not* rendered insensible after the initial shot, four were shot again with the captive bolt; however only one of these was rendered irrecoverably

insensible. The other three joeys still showed signs of sensibility after the second shot and were either shot again (n=2) or euthanased (n=1). The third shot resulted in insensibility; however, one of the two animals showed signs of returning to sensibility after one minute and was euthanased. The other four animals that were not initially rendered insensible were euthanased. For the current study, the acceptable captive bolt success rate for rendering pouch young instantaneously insensible was set at 95%. The observed success rate was 61% (13 successes out of 21 shots), which was significantly below the 95% threshold rate (Exact binomial test, P < 0.001). There was no association between age ($\chi^2 = 0.324$, df = 1, P = 0.569) or species ($\chi^2 = 1.54$, df = 2, P = 0.462) of joey with effectiveness of captive bolt. Also, there was no evidence that skull thickness ($\chi^2 = 2.65$, df = 1, P = 0.103) or the depth of bolt penetration ($\chi^2 = 1.68$, df = 1, P = 0.195) influenced effectiveness of the captive bolt. However, there was a significant relationship between position of shot and effectiveness at causing insensibility (Table 3). Barnard's test indicated that shots caudal to the crown were more effective than shots at the crown or rostral to the crown shot for producing insensibility (Wald's statistic = 2.037, twotailed P-value = 0.0496). Skull thickness at the captive bolt entrance cavity, bolt penetration depth and diameter of bolt entrance cavity were similar to that reported in the cadaver trials. Detailed assessment of damage to specific brain structures was not possible in many of the heads due to varying levels of post-mortem deterioration occurring from autolysis; freezing and thawing of the head; and confounding damage caused by multiple shots and secondary euthanasia with blunt trauma to the head. Consequently, it was not possible to relate damage to specific brain structures with clinical signs of insensibility. In the heads that could be examined (n=10), skull and brain damage varied depending on the trajectory of the bolt. The damage that was observed included: bolt wound tracts, extensive haemorrhage over the brain, herniation of the

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cerebellum, occipital lobe and cerebellum tissue extending towards the bolt entrance cavity, bone fragments in the region of the bone entrance cavity, and plugs of skin or hair pushed into the brain (Figures 2 and 3). Damage to different parts of the brain was assessed visually and graded (see Table 3). Logistic regression indicated that there was weak evidence that no macroscopic damage to the brain in general was associated with insensibility ($\chi^2 = 13.46$, df = 7, P = 0.062). There was no evidence that insensibility was associated with damage to any specific region of the brain. However, these analyses had low power owing to the small sample size available.

Performance of spring-powered captive bolt guns

The results of performance testing of two Dick KTBG and one Finito captive bolt guns are presented in Table 4 and Fig. 4. In Table 4, the results of a 0.22 Cash Special cartridge-powered captive bolt gun with the 1.0 and 1.25 gr powerloads are included for comparison. The mean \pm s.d. peak velocities (Finito: 8.77 \pm 0.24 m s⁻¹; Dick KTBG A: 9.14 \pm 0.62 m s⁻¹; and Dick KTBG B: 9.02 \pm 0.26 m s⁻¹) of the spring-powered captive bolt guns were lower than those of cartridge-powered 0.22 Cash Special with the 1.0 and 1.25 gr cartridges (velocity: 30.26 \pm 3.35 and 44.60 \pm 1.46 m s⁻¹, respectively) (F_{1,3} = 28.40, P = 0.0129). Additionally, the bolt weights (Finito: 102; and Dick KTBG: 120 g) of the spring-powered captive bolt guns were lower than the bolt weight of the Cash Special (211 g). Therefore, the spring-powered guns delivered a maximum kinetic energy of only 5.01 J, compared with the lowestpowered cartridge in the Cash Special delivering 97 J.

Of the two models of spring-powered guns, the Dick KTBG had the highest peak velocity, but the velocity decayed over the last 16–28 mm of recorded bolt travel. In comparison, the Finito had the lowest peak velocity, but the velocity was consistent through the full travel of the bolt (Fig. 4).

Discussion

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The study demonstrated that spring-powered penetrative captive bolt guns were ineffective at producing consistent, irrecoverable insensibility of in-pouch kangaroo joeys. Despite appearing to cause adequate damage to the brain when trialled on cadaver heads, a significant proportion of live animals were *not* irrecoverably concussed with a single shot. Although there was evidence of concussion in the majority of animals, 38% of animals still exhibited signs of sensibility after being shot. Therefore, on the basis of the guns tested, the relative effectiveness and humaneness of spring-powered captive bolt guns should be questioned as a method for stunning or killing of kangaroo pouch young. The success of captive bolt shooting for producing irrecoverable insensibility is dependent on delivering sufficient kinetic and direct physical damage to the brain (Daly & Whittington, 1989a; Gibson et al. 2012). This is influenced by factors relating to the captive bolt gun, animal and operator. Important captive bolt characteristics include velocity and captive bolt diameter. Studies in cattle, have reported that increasing bolt velocity during captive bolt stunning eliminates or reduces the incidence of recovery of visual evoked potentials (VEP), which are an indicator of brain function (Daly et al. 1987). Work by von Wenzlawowicz et al. (2012), suggested that for cattle shooting accuracy is less critical if high-powered captive bolt guns are used. Additionally, the transfer of kinetic energy to the head and the resulting depth of stun in cattle has been shown to improve with increasing bolt diameter (Gregory & Shaw 2000). In the current study, when trialled on cadavers, the Dick KTBG spring-powered captive bolt device appeared to cause sufficient physical trauma to areas of the brainstem, damage to which has been previously associated as being incompatible with maintenance of sensibility in humans and sheep (Adams and Graham 1986; Gibson et al. 2012). However, when trialled on live animals, it is possible that the device may not have had sufficient kinetic energy to irrecoverably concuss joeys. Furthermore, the bolt of the gun may have been too short (30

mm) or too narrow (4.7 mm) to produce the required trauma to cause irrecoverable concussion, especially for misplaced shots. Velocity of the Dick KTBG captive bolt gun was variable over the last 16 to 28 mm of travel of the bolt (Figure 1), which may have resulted in insufficient energy being transferred to brain. The kinetic energy of the Dick KTBG (4.9J) was 20 times less than what of the .22 Cash Special (97J) with its lowest strength cartridge, and this cartridge strength is only recommended for the dispatch of poultry (Table 1). The spring-powered captive bolt guns tested in this study were chosen based on their practicality, low cost (AUD \$65-85 per device), simplicity to operate and maintain, their small size and light weight, thus allowing shooters to carry them in the field. However, poor effectiveness on live animals along with low performance characteristics (especially when compared with other devices) should preclude them from being used on kangaroo in-pouch joeys. In addition to bolt characteristics, other factors such as bolt placement, type of animal, age, size and shape of head, skull anatomy including thickness, density of bone and calcification can all influence the aiming of the shot and the effectiveness of captive bolt stunning and dispatch (Finnie et al. 2003; Gouveia et al. 2009; Gregory & Shaw 2000). The ideal shooting position in the head can vary depending on species, however, prior to this study there had been no previous research to determine the ideal placement of the shot in kangaroos. Slaughter guidelines state that the optimum position for most animals is where the brain is closest to the surface of the head and where the skull is thinnest (Humane Slaughter Association 2006). Thus, based on the findings from the cadaver skulls, it is theorised that the ideal shooting position was at the highest point on the head (i.e. the crown) at the midline, where the skull is only around 1mm thick. Damage to the thalamus, and brainstem has been previously associated with irrecoverable insensibility in sheep (Gibson et al. 2012). In the current study, incorrect shot placement may have resulted in insufficient damage to these vital brain structures. Although all shots on cadavers and live animals were aimed at the crown of the head on the midline, the actual path of the bolt was variable. However, it was observed that shots caudal to the crown were more effective at inducing insensibility (100%, n=4)

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compared with shots at the crown or rostral to the crown (42%, n=5). These results indicate that the caudal shots were likely to result in damage to the thalamus and brainstem. However, as the trials with live animals were conducted under field conditions (i.e. at night, in remote locations with limited access to refrigerated storage for specimens), damage to specific brain regions, in terms of gross pathology, could not be examined in detail. Therefore, the relationship between brain damage severity and clinical signs of sensibility/insensibility could not be examined.

Effective captive bolt stunning is dependent on the accurate placement of the shot, operator skill and experience. Good marksmanship has been found to be a definitive factor in effective and humane use of captive bolt guns for the irrecoverable dispatch of sheep without a secondary procedure (Gibson et al. 2012).

Properties of the skull and brain of immature animals could also potentially influence the effectiveness of captive bolt stunning. Insensibility from penetrating captive bolt stunning is caused by a combination of direct mechanical damage to the brain (diencephalon and brainstem) by the penetrating bolt and focal and diffuse injuries to the white matter pathways connecting these areas (Finnie *et al.* 2002). Much of this diffuse damage is thought to occur during the biomechanical transfer of kinetic energy from the bolt to head at the time of impact (Shaw 2002). When the bolt impacts the skull it produces a rapid acceleration of the head resulting in contre-coup, sear forces and the transferring of pressure waves within the brain and cranial vault (Anderson and McLean 2005). Daly & Whittington (1989) have argued that the *main* cause of effective stunning is this transfer of kinetic energy from the bolt to the cranial vault as opposed to the direct physical damage caused by the bolt. In very young animals where the skull has not fully ossified (or hardened), it is possible that the energy from the bolt impacting the cranium could be dissipated though the skull prior to being transferred to the brain. This could result in incomplete or inadequate concussion. Concern about the

effectiveness of captive bolt guns for stunning young livestock (lambs, goat kids, and newborn calves) has been previously raised (e.g. Svendsen et al. 2008; Schutt-Abraham and Wormuth, 1995 cited in EFSA AHAW panel 2006). However, some studies have demonstrated that both penetrating and non-penetrating captive bolt guns are effective in causing immediate insensibility in young livestock (e.g. Gibson et al. 2009; Finnie et al. 2000). Svendsen et al. (2008), in a study of one-day-old calves, reported that all animals were rendered immediately insensible after penetrative captive bolt gun stunning. However, unlike neonates of placental mammals, marsupials are born relatively developmentally immature, with much of the development occurring in the pouch. The skulls of in-pouch joeys are softer and less ossified than neonates of other livestock species. Gregory (2007) suggested during captive bolt stunning of young rabbits that if the bolt strikes a skull suture there could be a higher risk of poor stunning. This could be due to some of the kinetic energy from the bolt being absorbed by the un-fused skull suture. Therefore, the skulls of developmentally immature animals (such as in-pouch kangaroo young) may possibly inhibit the energy transfer from the bolt to the brain, making these animal more difficult to concuss with a captive bolt devices compared with older animals which have much harder skulls and fused sutures. Furthermore, the shear forces and inertial loading experienced during captive bolt trauma are related to brain mass. It has been shown that animals with smaller brains can tolerate greater rotational and acceleration/deceleration forces than humans and non-human primates (Ommaya et al 1967). Further work is needed before dispatch by captive bolt can be considered as a humane and acceptable alternative to the currently used manually applied blunt trauma to the head. Additional studies could be performed to examine the relationship between the pathophysiology of captive bolt injury in joeys and behaviour/brainstem-mediated signs of CNS function or dysfunction. This was not possible in the current project, due to the majority of the work been conducted under field conditions. Furthermore, the effectiveness of other models of captive bolt guns could be examined. This could include the cartridge powered

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captive bolt guns (Cash Specials, Cash Poultry Killer), which have higher peak velocity and kinetic energy values. In addition, the effects of captive bolt shooting on brain function using either changes in the spontaneous electroencephalogram (EEG) or somatosensory/visual-evoked potentials could be examined in joeys of different ages. This would provide useful information on the effect of age of the joey on captive bolt effectiveness and provide a more objective measure of altered brain function following captive bolt injury.

In conclusion, it was found that *spring-powered* penetrative captive bolt guns, although practical to use, were ineffective in consistently rendering in-pouch kangaroo joeys irrecoverably insensible. Animals that were incompletely concussed or recovered sensibility could have experienced pain and distress associated with captive bolt injury. Based on these findings, dispatch by spring-powered captive bolt cannot be considered a humane and acceptable alternative to the currently used method of manually applied blunt trauma to the

head.

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Table 1. Effectiveness of a spring-powered captive bolt gun on three different species of kangaroo pouch young

Failed stun, the animal was not renderedinsensible by the initial shot. Immediately insensible but recovered, animal showedimmediate insensibility after the initial shot then regained sensibility after >1 min. Irrecoverably insensible, the animal showed immediate insensibility after the initial shot and did not regain consciousness (i.e. died after 1 min or was euthanased after stunning, using a secondary euthanasia method)

	Effectiveness of captive bolt shot				
Species (mean ± SD age)	Failed stun (%) ^a	Immediately insensible but recovered (%) ^b	Irrecoverably insensible (%) ^c		
Red kangaroo (Macropus rufus) (195 ± 2 d)	4 (50%)	0 (0%)	4 (50%)		
Eastern grey kangaroo (Macropus giganteus) (253 ± 40 d)	4 (33%)	4 (33%)	4 (33%)		
Western grey kangaroo (Macropus fuliginosus) (166 d)	0 (0%)	0 (0%)	1 (100%)		

^aFailed stunned = was not rendered insensible by initial shot

^bImmediate insensibility after initial shot then regained sensibility after > 1 minute

^cImmediate insensibility after initial shot and did not regain consciousness (i.e. died after 1 minute or was euthanased after stunning using a secondary euthanasia method).

Table 2. Influence of the position on the head on the effectiveness of stunning in live pouch young, using a spring-powered captive bolt

Failed stun, the animal was not rendered insensible by the initial shot. Irrecoverably insensible, the animal showed immediate insensibility after the initial shot and did not regain consciousness (i.e. died after 1 min or was euthanased after stunning, using a secondary euthanasia method)

	Effectiveness of captive bolt shot			
Position of shot	Irrecoverably insensible	Failed stun		
At crown or in front of crown (rostral)	5 (42%)	7 (58%)*		
Behind crown (caudal)	4 (100%)	0 (0%)		

^{*}Note: for one animal the position of the first shot could not be determined

Table 3. Macroscopic assessment of damage to different brain areas from spring-powered captive bolt in live kangaroo pouch young

Damage to the left and right lobes of the cerebrum were grouped to aid analysis. There was no recorded damage to the spinal cord. +++, severe; ++, moderate; +, mild; -, none

	Macroscopic structural damage to:									
Immediate insensibility	Thalamus	Midbrain	Pons	Medulla	Cerebellum	Frontal lobe	Parietal lobe	Temporal lobe	Occipital lobe	
Yes	-	-	+++	++	+++	-	-	-	-	
Yes	-	+	+	-	-	-	++	+	++	
Yes	+	+++	-	-	-	-	-	+++	-	
Yes	+	-	-	-	-	++	++	-	-	
No	-	-	-	-	-	+	+	-	-	
No	-	-	-	-	+*	+++	-	-	-	
No	+	-	-	-	+*	-	+++	-	+	
No	-	-	-	-	+*	++	++	-	-	
Yes	-	-	-	-	-	+++	-	-	-	
Yes	+	-	-	-	+*	+++	++	-	-	
Yes	+	-	-	-	+*	+++	++	-		

^{*} Damage in the form of cerebral coning

Note: Damage to the left and right lobes of the cerebrum were grouped to aid analysis. No recorded damage to the spinal cord.

Table 4. Captive bolt features, mode of action, peak velocity, kinetic energy and penetration depth. Results from a .22 Cash Special are included here for comparison.

Captive	Cartridge/power	Bolt	Nominal	Mean peak	Velocity range	Kinetic	Penetration
bolt	source	Weight (g)	propellant velocity ± SD		(m.s ⁻¹)	energy	depth <u>+</u> SD
			charge (mg)	(m.s ⁻¹)	(m.s)	(J)	(mm)
.22 Cash	1.0 gr Clear	211	110	30.26 <u>+</u> 3.34	24.10 – 34.60	97	63 <u>+</u> 1
Special	1.25 gr Pink	211	170	44.60 <u>+</u> 1.46	41.40 – 45.80	210	68 <u>+</u> 2
Finito	Spring	102	n/a	8.77 ± 0.24	8.20 – 9.20	3.92	25.66 ± 0.70
Dick KTBG A	Spring	120	n/a	9.14 <u>+</u> 0.62	8.60 – 12.70	5.01	27.49 <u>+</u> 1.83
Dick KTBG B	Spring	120	n/a	9.02. ± 0.26	8.40 – 9.40	4.88	28.31 ± 0.88

Figures





Fig. 1. Top- Dick KTBG spring-powered captive bolt gun (source Friedr. Dick GmbH & Co. KG, Germany). **Bottom-** Finito spring-powered captive bolt gun (source Klaus-Gritsteinwerk GmbH & Co, Bünde, Germany)

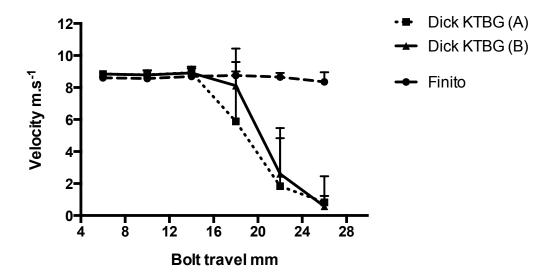


Fig. 2. Peak velocity measurements for two Dick KTBG and one Finito bolt guns. There is variability between the two Dick KTBG guns and decay of velocity over the last 16 to 28 mm of recorded bolt travel. With the Finito captive bolt gun there is less decay of velocity over the last 16 to 28 mm of recorded bolt travel compared with the Dick KTGB guns.



Fig. 2. Sagittal section of a head from a shot that was too far rostral. This animal was rendered insensible but regained sensibility. The bolt did not appear to pass into the midbrain or brain stem but was closer to the olfactory cortex.



Fig. 3.a. Sagittal section of a head from a shot slightly rostral to the top of the head. This animal was rendered insensible and did not regain sensibility after 4 minutes, after which time it was euthansed. A fragment of skull bone has been pushed into brain by the bolt.



Fig. 3.b View of top of the head (same animal as in Figure 3) showing bolt hole rostral to the crown and to the right of the midline.

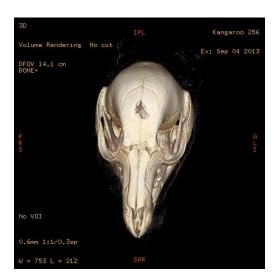




Fig. 3.c. 3D CT reconstruction of animal from Figures 3.a and b.

Left - Frontal view showing hole and fracture caused by the bolt (note position rostral to the crown)

Right - Cut away view of inside skull showing a fragment of bone has been pushed inside the skull by the bolt.

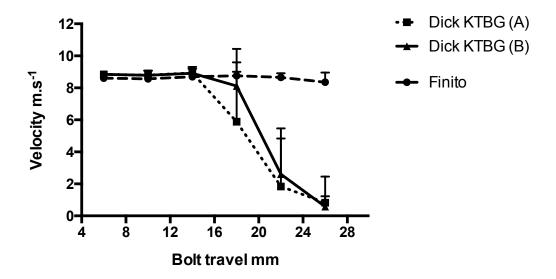


Fig 4. Peak velocity measurements for two Dick KTBG and one Finito bolt guns. There is variability between the two Dick KTBG guns and decay of velocity over the last 16 to 28 mm of recorded bolt travel. With the Finito captive bolt gun there is less decay of velocity over the last 16 to 28 mm of recorded bolt travel compared with the Dick KTGB guns.