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2 3	Evaluation of spring-powered captive bolt guns for dispatch of kangaroo in-pouch young
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31 Abstract

- 32 *Context*. During commercial harvesting or non-commercial kangaroo culling
- programs, furred pouch young of shot females are required to be euthanased to
- 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.
- 37 *Aims*. To determine if an alternative method for dispatching pouch young— a spring-
- 38 operated captive bolt gun—is practical and effective at causing immediate
- 39 insensibility in kangaroo pouch young.
- 40 *Methods.* Trials of the spring-operated captive bolt guns were conducted first on the
- 41 heads of pouch young cadavers and then on live pouch young, during commercial
- 42 harvesting. Performance characteristic of the spring-operated guns were also
- 43 measured and compared with cartridge-powered devices.
- *Key results.* The captive bolt guns caused insensibility in only 13 out of 21 trials on
 live pouch young. This 62% success rate is significantly below the 95% minimum
 acceptable threshold for captive bolt devices in domestic animal abattoirs. Failure to
 stun was related to bolt placement, but other factors such as bolt velocity, bolt
 diameter and skull properties such as density might have also contributed. Springoperated captive bolt guns delivered 20 times less kinetic energy when compared with
 cartridge-powered devices.
- *Conclusions.* Spring-operated captive bolt guns cannot be recommended as an
 acceptable or humane method for dispatching kangaroo pouch young.
- *Implications.* Captive bolts guns have potential as a practical alternative to blunt head
 trauma that may standardise dispatch technique and reduce animal (and observer)
 distress. However, operators must continue to use the existing prescribed dispatch
 methods until cartridge-powered captive bolt guns have been trialled as an alternative
 bolt propelling method.
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59 Keywords: kangaroo harvesting, captive bolt gun, culling, euthanasia, blunt trauma,

- 60 animal welfare, humaneness, joey
- 61

62 Introduction

63 In Australia, all states and territories have legislation to protect kangaroos, however, under 64 strict government control, four of the most abundant species are harvested commercially (by 65 shooting) for meat and skin products. Kangaroos are also shot during non-commercial culling 66 to reduce population size and thereby reduce negative impacts on the environment or 67 agricultural production. Commercial and non-commercial shooting differ in that commercial 68 shooters must be licensed and require a higher level of training compared with non-69 commercial shooters. Also, commercial harvesting must be done in accordance with a 70 government approved management plan and compliance with a code of practice (Anon 71 2008a) is monitored.

72 Minimum animal welfare standards for both commercial and non-commercial shooting of 73 kangaroos are prescribed in national codes of practice (Anon 2008a; Anon 2008b). Both 74 codes require that dependent young of shot females must be euthanased to prevent them from 75 suffering. Specified acceptable euthanasia methods for small, furless pouch young (i.e. that fit 76 within the palm of the hand) are either a 'single forceful blow to the base of the skull 77 sufficient to destroy the functional capacity of the brain' or 'stunning, immediately followed 78 by decapitation by rapidly severing the head from the body with a sharp blade'. Furred pouch 79 young must be dispatched by a 'single forceful blow to the base of the skull sufficient to 80 destroy the functional capacity of the brain'. Although the codes of practice do not provide 81 specific guidelines on how to apply the single forceful blow to the head, commercial 82 kangaroo shooters usually do this by holding the joey by the hindquarters and swinging it in 83 an arc so that its head hits a hard object such as a large rock or side of the rack or tray on their 84 vehicle. Larger furless joeys are sometimes placed onto the ground and the head is stomped 85 on with the foot and occasionally shooters use a heavy bar or pipe to hit the joey on the head 86 whilst holding them by the back-legs (McLeod and Sharp in press). All of these procedures fit 87 within the codes' loose definition of a 'single forceful blow to the head' as described

88 in the Code (Anon 2008a; Anon 2008b).

89 According to international guidelines on euthanasia, manually applied blunt trauma to the 90 head can be a rapid and humane method of dispatching small animals such as birds, 91 amphibians, fish, reptiles and some neonatal animals with thin skulls (e.g. pigs) (AVMA 92 Panel on Euthanasia 2013). However, to be effective and humane, the method must be applied 93 using a single sharp blow delivered to the cranium with sufficient force to produce immediate 94 depression of CNS (central nervous system) function and destruction of brain tissue, 95 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 96 97 unsightly and emotionally unpleasant for both observers and operators. There is also a 98 reluctance of some operators to perform dispatch by blunt force trauma. When dispatching 99 joevs, if the operator does not deliver the blow with sufficient force or does not contact the 100 correct position on the head, then there is the potential that the animal will not be rendered 101 completely insensible and it could experience pain and distress. Some guidelines consider 102 blunt trauma to be only acceptable in instances where it is the most rapid and practical 103 method available (e.g. for the emergency euthanasia of injured newborn piglets, CCAC 104 2010). Experts on euthanasia have also recommended that blunt trauma should be replaced 105 when possible with alternative methods (AVMA Panel on Euthanasia 2013). However, some 106 of the alternatives suggested are not suitable for use on wild animals in field situations. For 107 example, it has been proposed that, during harvesting, joeys should be euthanased with a 108 lethal injection administered by a veterinarian (NSW Young Lawyers Animal Law 109 Committee 2008 cited in Boom and Ben-Ami 2011). This would involve distress and pain 110 associated with handling, restraint and the injection. Also, it would be impractical and 111 expensive to carry out and there would be negative consequences for non-target animals that 112 scavenge carcasses that are not disposed of correctly.

113 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

115 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 116 describing culling methods using emotionally charged language, for example, 'Orphaned 117 joeys face a bloody and barbaric death' (Holland 2009). A recent survey showed that the 118 119 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 120 121 Animals (RSPCA) has also questioned the appropriateness of the techniques prescribed for 122 dispatching pouch young and proposed that research should be urgently conducted to 123 determine what methods are the most humane (RSPCA Australia 2002; 2009b).

124 A potential alternative to blunt trauma would be the dispatch of joeys with captive bolt guns.

125 Captive bolt guns fire a steel bolt that either penetrates (*penetrating captive bolt*) or impacts

126 (*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

129 gunpowder cartridges, compressed air or a spring mechanism.

130 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 131 132 recovery. For example, when cattle are slaughtered for human consumption, they are often 133 stunned with a captive bolt gun and then exsanguinated. However, it has also been reported 134 that captive bolt devices can be used as a single-step method for killing cattle (Gilliam et al. 135 2012) and sheep (Gibson et al. 2012) without the need for sticking or pithing, when shot in 136 the correct position. Although, mostly used for the stunning of larger animals (sheep and 137 cattle), captive bolt guns have also been developed for use on smaller animals including 138 poultry (Raj & O'Callaghan 2001), dogs (Dennis et al. 1988) and rabbits (Holtzmann 1991). 139 The recommended stunning positions vary widely between species, principally due to 140 differences in the anatomy of the head and skull. In rabbits, the currently recommended 141 stunning position is on the top of the head at the midline between the base of the ears

142 (Holtzmann 1991; EFSA AHAW panel 2006). There have been no studies on the use of 143 captive bolt guns for the stunning or the killing of kangaroo pouch young. When blunt trauma 144 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 145 146 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. 147 148 Spring-powered captive bolt devices, which are used to stun small animals such as rabbits and 149 poultry, are compact and portable, and so would be convenient for using in field situations. 150 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 151 152 624 Wildlife Research T. M. Sharp et al. to own or operate, as is the case in some states in 153 Australia. Informal discussions with harvesters and a representative of the NSW Kangaroo 154 Management Agency (NSW Office of Environment and Heritage, Kangaroo Management 155 Section) before the present study indicated a preference for testing the spring-powered 156 devices because of these advantages. Thus, the aim of the study was to determine whether 157 commercially available spring-powered penetrative captive bolt guns are effective for the killing of pouch young during commercial harvesting or non-commercial culling of 158 159 kangaroos.

160 Materials and Methods

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 onthe heads of carcasses. These were the Dick KTBG (Friedr. Dick GmbH and Co, Deizisau,

167 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.

173 With the tests in dead animals, we assessed the degree of skull and brain damage caused by 174 the bolt and also examined skull properties such as thickness. This information was then used 175 to determine potential captive bolt placement sites, with the aim of causing extensive damage 176 to specific brain structures (cerebral cortex and brainstem). We then assessed the 177 effectiveness of the spring-powered captive bolt in causing irrecoverable insensibility in live 178 animals. An accepted welfare standard in livestock abattoirs is that the first shot must 179 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, 180 181 kinetic energy, penetration depth) of spring-powered captive bolt guns were also examined in 182 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. 183

184 Trials on cadaver heads

185 In total, 15 heads from dead eastern grey kangaroo (Macropus giganteus) young were used to 186 examine the penetration characteristics of the captive bolt guns and macroscopic damage to 187 skull and brain structures. Ten of the carcasses were sourced from veterinary clinics, and five 188 were obtained from commercial kangaroo shooters. The origin of every animal was not 189 known; however, most of those from the veterinary clinics had been euthanased with an 190 injection of barbiturate because of sickness or injury and some had been found dead as a 191 result of trauma from a collision with a motor vehicle. The animals from the shooters had 192 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 forstorage at -20C and defrosted for 18–24 h before testing.

195 One operator performed all of the trials on the cadaver heads. Each head was shot once on the 196 highest point of the head at the midline, with the gun held at a perpendicular angle to the 197 skull. After firing, the skulls were skinned and the position, shape and size of the bolt 198 entrance cavity on the cranium recorded. Trajectory and penetration depth of the bolt was 199 measured from the outer surface of the skulls using a wooden probe inserted through the bolt 200 entrance cavity. The heads were sawed (with a hacksaw) longitudinally through or near to the 201 bolt penetration site. The skull, brain and specific brain structures were visually assessed. 202 Skull thickness at various points was measured and damage to the brain was recorded using 203 digital photographs. Skull thickness and bolt penetration depth were measured using digital 204 vernier calipers (JBS tools).

205 *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.

225 Immediately after shooting, all animals were examined for clinical signs of insensibility 226 including sudden loss of muscle tone (body going limp), lack of purposeful or coordinated 227 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 228 229 rhythmic breathing and a heartbeat were also noted for each animal. Instantaneous 230 insensibility after one shot was scored as a successful (or effective) shot, while any sign of 231 sensibility was scored as unsuccessful (or ineffective) shot. Animals effectively stunned were 232 observed for 5 min and time to recovery or death was noted. Animals not effectively stunned 233 were immediately re-shot or euthanased. When euthanasia was performed, it was done by 234 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.

242 Performance of spring-powered captive bolt guns

243 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,

245 Bedford, UK). The meter measured velocity of the bolt as it transects a series of seven 246 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 247 bolt guns were fired 40 times for velocity assessment using the meter. Peak velocity was 248 249 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, 250 Acculab UK, Sartorius Group, Epson, Surrey, UK). Peak velocity of the bolt was recorded 251 and used to calculate the kinetic energy of the bolt (*Kinetic energy* = $(\frac{1}{2} \times m) \times v^2$; where m 252 = mass of the bolt (kg) and v = peak velocity (m.s⁻¹)). By determining the kinetic energy, the 253 two different captive bolt gun models were compared whilst taking into account differences in 254 255 bolt weight. Peak velocity of the spring-powered captive bolt guns was compared with those 256 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 257 258 al. submitted). Penetration depth was measured with the firing of the captive bolt guns into 259 ballistics gelatine moulds. Five shots were fired 30 mm apart with the mean penetration depth 260 calculated. The ballistics gelatine was prepared according to Fackler and Malinowski (1988). 261 The diameter and length of the Dick KTBG bolt was 4.7 and 30 mm respectively, while for 262 the Finito it was 5.4 and 33 mm respectively.

263 Statistical analysis

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. 271 The R package 'Barnard', version 1.3 (Erguler 2012), was used to perform Barnard's 272 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 273 from a position either at the crown/in front of the crown (rostral), or behind the crown 274 275 (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 276 pouch young insensible. We first used the R function 'glm' (R Core Team 2014) to fit full 277 278 and nested generalised linear models to these data, specifying a binomial error distribution. 279 The significance of the independent variables was determined by comparing the full and 280 nested models with the restricted model, by using the likelihood-ratio test. In addition, the 281 relationship between insensibility and damage to specific brain areas was also examined using 282 logistic regression.

283 **Results**

284 Trials on cadaver heads

285 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 $1.00 (\pm 0.32 \text{ SD})$ mm and the

mean bolt penetration depth was 27 (\pm 3.5 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 alsopushed into the wound tract with some heads.

298 When shooting in the crown position, we observed some cases of skin slippage', the 299 movement of the skin across the underlying skull (Gregory 2007, pp. 196). This resulted in 300 the bolt being misplaced, to the right or left of the midline and/or to the front (rostral) or to 301 the back (caudal) of the crown. If skin slippage occurs during shooting of live animals, it 302 could cause the captive bolt to enter the brain at the incorrect position, potentially resulting in 303 incomplete concussion. To minimise the risk of skin slippage, the muzzle of the captive bolt 304 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 305 gun before and during discharge. 306

307 *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 wereshot 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 330 = 2, P = 0.462) of joey with effectiveness of captive bolt. Also, there was no evidence that 331 skull thickness ($\chi^2 = 2.65$, df = 1, P = 0.103) or the depth of bolt penetration ($\chi^2 = 1.68$, df = 1, 332 P = 0.195) influenced effectiveness of the captive bolt. However, there was a significant 333 relationship between position of shot and effectiveness at causing insensibility (Table 3). 334 335 Barnard's test indicated that shots caudal to the crown were more effective than shots at the 336 crown or rostral to the crown shot for producing insensibility (Wald's statistic = 2.037, twotailed P-value = 0.0496). 337

338 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 339 340 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 341 head; and confounding damage caused by multiple shots and secondary euthanasia with blunt 342 343 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), 344 skull and brain damage varied depending on the trajectory of the bolt. The damage that was 345 346 observed included: bolt wound tracts, extensive haemorrhage over the brain, herniation of the

347 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 348 the brain (Figures 2 and 3). Damage to different parts of the brain was assessed visually and 349 350 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 = 351 7, P = 0.062). There was no evidence that insensibility was associated with damage to any 352 specific region of the brain. However, these analyses had low power owing to the small 353 sample size available. 354

355 *Performance of spring-powered captive bolt guns*

356 The results of performance testing of two Dick KTBG and one Finito captive bolt guns are

357 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 ± 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

362 (velocity: 30.26 ± 3.35 and 44.60 ± 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

364 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).

371 **Discussion**

The study demonstrated that spring-powered penetrative captive bolt guns were ineffective at 372 373 producing consistent, irrecoverable insensibility of in-pouch kangaroo joeys. Despite 374 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 375 there was evidence of concussion in the majority of animals, 38% of animals still exhibited 376 377 signs of sensibility after being shot. Therefore, on the basis of the guns tested, the relative 378 effectiveness and humaneness of spring-powered captive bolt guns should be questioned as a 379 method for stunning or killing of kangaroo pouch young.

The success of captive bolt shooting for producing irrecoverable insensibility is dependent on 380 381 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, 382 383 animal and operator. Important captive bolt characteristics include velocity and captive bolt 384 diameter. Studies in cattle, have reported that increasing bolt velocity during captive bolt 385 stunning eliminates or reduces the incidence of recovery of visual evoked potentials (VEP), 386 which are an indicator of brain function (Daly et al. 1987). Work by von Wenzlawowicz et al. 387 (2012), suggested that for cattle shooting accuracy is less critical if high-powered captive bolt 388 guns are used. Additionally, the transfer of kinetic energy to the head and the resulting depth 389 of stun in cattle has been shown to improve with increasing bolt diameter (Gregory & Shaw 2000). 390

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

397 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 398 variable over the last 16 to 28 mm of travel of the bolt (Figure 1), which may have resulted in 399 400 insufficient energy being transferred to brain. The kinetic energy of the Dick KTBG (4.9J) 401 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 402 403 spring-powered captive bolt guns tested in this study were chosen based on their practicality, 404 low cost (AUD \$65-85 per device), simplicity to operate and maintain, their small size and 405 light weight, thus allowing shooters to carry them in the field. However, poor effectiveness on 406 live animals along with low performance characteristics (especially when compared with 407 other devices) should preclude them from being used on kangaroo in-pouch joeys. 408 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 409

411 dispatch (Finnie *et al.* 2003; Gouveia *et al.* 2009; Gregory & Shaw 2000). The ideal shooting

can all influence the aiming of the shot and the effectiveness of captive bolt stunning and

412 position in the head can vary depending on species, however, prior to this study there had

413 been no previous research to determine the ideal placement of the shot in kangaroos.

410

414 Slaughter guidelines state that the optimum position for most animals is where the brain is

415 closest to the surface of the head and where the skull is thinnest (Humane Slaughter

416 Association 2006). Thus, based on the findings from the cadaver skulls, it is theorised that the

417 ideal shooting position was at the highest point on the head (i.e. the crown) at the midline,

418 where the skull is only around 1mm thick. Damage to the thalamus, and brainstem has been

419 previously associated with irrecoverable insensibility in sheep (Gibson *et al.* 2012). In the

420 current study, incorrect shot placement may have resulted in insufficient damage to these vital

421 brain structures. Although all shots on cadavers and live animals were aimed at the crown of

422 the head on the midline, the actual path of the bolt was variable. However, it was observed

423 that shots caudal to the crown were more effective at inducing insensibility (100%, n=4)

424 compared with shots at the crown or rostral to the crown (42%, n=5). These results indicate 425 that the caudal shots were likely to result in damage to the thalamus and brainstem. However, 426 as the trials with live animals were conducted under field conditions (i.e. at night, in remote 427 locations with limited access to refrigerated storage for specimens), damage to specific brain 428 regions, in terms of gross pathology, could not be examined in detail. Therefore, the 429 relationship between brain damage severity and clinical signs of sensibility/insensibility could 430 not be examined.

431

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).

436 Properties of the skull and brain of immature animals could also potentially influence the 437 effectiveness of captive bolt stunning. Insensibility from penetrating captive bolt stunning is 438 caused by a combination of direct mechanical damage to the brain (diencephalon and 439 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 440 during the biomechanical transfer of kinetic energy from the bolt to head at the time of impact 441 442 (Shaw 2002). When the bolt impacts the skull it produces a rapid acceleration of the head 443 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 444 445 the *main* cause of effective stunning is this transfer of kinetic energy from the bolt to the 446 cranial vault as opposed to the direct physical damage caused by the bolt. In very young 447 animals where the skull has not fully ossified (or hardened), it is possible that the energy from 448 the bolt impacting the cranium could be dissipated though the skull prior to being transferred 449 to the brain. This could result in incomplete or inadequate concussion. Concern about the

450 effectiveness of captive bolt guns for stunning young livestock (lambs, goat kids, and 451 newborn calves) has been previously raised (e.g. Svendsen et al. 2008; Schutt-Abraham and 452 Wormuth, 1995 cited in EFSA AHAW panel 2006). However, some studies have 453 demonstrated that both penetrating and non-penetrating captive bolt guns are effective in 454 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 455 456 rendered immediately insensible after penetrative captive bolt gun stunning. However, unlike neonates of placental mammals, marsupials are born relatively developmentally immature, 457 with much of the development occurring in the pouch. The skulls of in-pouch joevs are softer 458 and less ossified than neonates of other livestock species. Gregory (2007) suggested during 459 460 captive bolt stunning of young rabbits that if the bolt strikes a skull suture there could be a 461 higher risk of poor stunning. This could be due to some of the kinetic energy from the bolt 462 being absorbed by the un-fused skull suture. Therefore, the skulls of developmentally 463 immature animals (such as in-pouch kangaroo young) may possibly inhibit the energy transfer 464 from the bolt to the brain, making these animal more difficult to concuss with a captive bolt 465 devices compared with older animals which have much harder skulls and fused sutures. 466 Furthermore, the shear forces and inertial loading experienced during captive bolt trauma are 467 related to brain mass. It has been shown that animals with smaller brains can tolerate greater 468 rotational and acceleration/deceleration forces than humans and non-human primates 469 (Ommaya et al 1967).

470 Further work is needed before dispatch by captive bolt can be considered as a humane and471 acceptable alternative to the currently used manually applied blunt trauma to the head.

472 Additional studies could be performed to examine the relationship between the

473 pathophysiology of captive bolt injury in joeys and behaviour/brainstem-mediated signs of

474 CNS function or dysfunction. This was not possible in the current project, due to the majority

475 of the work been conducted under field conditions. Furthermore, the effectiveness of other

476 models of captive bolt guns could be examined. This could include the cartridge powered

477 captive bolt guns (Cash Specials, Cash Poultry Killer), which have higher peak velocity and 478 kinetic energy values. In addition, the effects of captive bolt shooting on brain function using 479 either changes in the spontaneous electroencephalogram (EEG) or somatosensory/visual-480 evoked potentials could be examined in joeys of different ages. This would provide useful 481 information on the effect of age of the joey on captive bolt effectiveness and provide a more 482 objective measure of altered brain function following captive bolt injury. 483 In conclusion, it was found that *spring-powered* penetrative captive bolt guns, although 484 practical to use, were ineffective in consistently rendering in-pouch kangaroo joeys irrecoverably insensible. Animals that were incompletely concussed or recovered sensibility 485 could have experienced pain and distress associated with captive bolt injury. Based on these 486 findings, dispatch by spring-powered captive bolt cannot be considered a humane and 487 488 acceptable alternative to the currently used method of manually applied blunt trauma to the head. 489

490

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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 \pm 2 d)	4 (50%)	0 (0%)	4 (50%)		
Eastern grey kangaroo (<i>Macropus giganteus</i>) (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	+	-	-	-	+*	+++	++	-	-

* 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 <u>+</u> SD (m.s ⁻¹)	(m.s ⁻¹)	energy	depth <u>+</u> SD
			charge (mg)			(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 <u>+</u> 0.24	8.20 - 9.20	3.92	25.66 <u>+</u> 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. <u>+</u> 0.26	8.40 - 9.40	4.88	28.31 <u>+</u> 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.