

## THE UNIVERSITY of EDINBURGH

## Edinburgh Research Explorer

# Evaluation of the potential killing performance of novel percussive and cervical dislocation tools in chicken cadavers

#### Citation for published version:

Martin, J, McKeegan, DEF, Sparrey, J & Sandilands, V 2017, 'Evaluation of the potential killing performance of novel percussive and cervical dislocation tools in chicken cadavers', *British Poultry Science*, vol. 58, no. 3, pp. 216-223. https://doi.org/10.1080/00071668.2017.1280724

#### Digital Object Identifier (DOI):

10.1080/00071668.2017.1280724

#### Link:

Link to publication record in Edinburgh Research Explorer

**Document Version:** Peer reviewed version

Published In: British Poultry Science

#### General rights

Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

#### Take down policy

The University of Édinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.



<ul> <li>cervical dislocation tools in chicken cadavers</li> <li>Jessica E Martin<sup>1,2,3*</sup>, Dorothy E F McKeegan<sup>3</sup>, Julian Sparrey<sup>4</sup> and Victoria Sandilands</li> <li><sup>1</sup>Animal Behaviour and Welfare, Animal and Veterinary Science Research Group, SRU</li> <li>Roslin Institute Building, Easter Bush, Edinburgh, EH25 9RG, UK</li> <li><sup>2</sup> The Royal (Dick) School of Veterinary Studies and The Roslin Institute, Easter Bush</li> <li>Campus, Edinburgh, Midlothian, EH25 9RG</li> <li><sup>3</sup> Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medica</li> <li>Veterinary &amp; Life Sciences, University of Glasgow, G61 1QH, UK</li> <li><sup>4</sup> Livetec Systems Ltd, Building 52, Wrest Park, Silsoe, Bedford, MK45 4HS, UK</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> </ul>	s <sup>1</sup> IC, า
<ul> <li>Jessica E Martin<sup>1,2,3*</sup>, Dorothy E F McKeegan<sup>3</sup>, Julian Sparrey<sup>4</sup> and Victoria Sandilands</li> <li><sup>1</sup>Animal Behaviour and Welfare, Animal and Veterinary Science Research Group, SRU Roslin Institute Building, Easter Bush, Edinburgh, EH25 9RG, UK</li> <li><sup>2</sup> The Royal (Dick) School of Veterinary Studies and The Roslin Institute, Easter Bush Campus, Edinburgh, Midlothian, EH25 9RG</li> <li><sup>3</sup> Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medica Veterinary &amp; Life Sciences, University of Glasgow, G61 1QH, UK</li> <li><sup>4</sup> Livetec Systems Ltd, Building 52, Wrest Park, Silsoe, Bedford, MK45 4HS, UK</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> </ul>	s <sup>1</sup> JC, 1
<ul> <li>Jessica E Martin<sup>1,2,3*</sup>, Dorothy E F McKeegan<sup>3</sup>, Julian Sparrey<sup>4</sup> and Victoria Sandilands</li> <li><sup>1</sup>Animal Behaviour and Welfare, Animal and Veterinary Science Research Group, SRU Roslin Institute Building, Easter Bush, Edinburgh, EH25 9RG, UK</li> <li><sup>2</sup> The Royal (Dick) School of Veterinary Studies and The Roslin Institute, Easter Bush Campus, Edinburgh, Midlothian, EH25 9RG</li> <li><sup>3</sup> Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medica Veterinary &amp; Life Sciences, University of Glasgow, G61 1QH, UK</li> <li><sup>4</sup> Livetec Systems Ltd, Building 52, Wrest Park, Silsoe, Bedford, MK45 4HS, UK</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> </ul>	s <sup>1</sup> JC, 1
<ul> <li><sup>1</sup>Animal Behaviour and Welfare, Animal and Veterinary Science Research Group, SRU Roslin Institute Building, Easter Bush, Edinburgh, EH25 9RG, UK</li> <li><sup>2</sup>The Royal (Dick) School of Veterinary Studies and The Roslin Institute, Easter Bush Campus, Edinburgh, Midlothian, EH25 9RG</li> <li><sup>3</sup>Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medica Veterinary &amp; Life Sciences, University of Glasgow, G61 1QH, UK</li> <li><sup>4</sup>Livetec Systems Ltd, Building 52, Wrest Park, Silsoe, Bedford, MK45 4HS, UK</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> </ul>	JC, 1 al,
<ul> <li>Roslin Institute Building, Easter Bush, Edinburgh, EH25 9RG, UK</li> <li><sup>2</sup> The Royal (Dick) School of Veterinary Studies and The Roslin Institute, Easter Bush Campus, Edinburgh, Midlothian, EH25 9RG</li> <li><sup>3</sup> Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medica Veterinary &amp; Life Sciences, University of Glasgow, G61 1QH, UK</li> <li><sup>4</sup> Livetec Systems Ltd, Building 52, Wrest Park, Silsoe, Bedford, MK45 4HS, UK</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> </ul>	ר זו,
<ul> <li><sup>2</sup> The Royal (Dick) School of Veterinary Studies and The Roslin Institute, Easter Bush Campus, Edinburgh, Midlothian, EH25 9RG</li> <li><sup>3</sup> Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medica Veterinary &amp; Life Sciences, University of Glasgow, G61 1QH, UK</li> <li><sup>4</sup> Livetec Systems Ltd, Building 52, Wrest Park, Silsoe, Bedford, MK45 4HS, UK</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> </ul>	ר זו,
<ul> <li>Campus, Edinburgh, Midlothian, EH25 9RG</li> <li><sup>3</sup> Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical Veterinary &amp; Life Sciences, University of Glasgow, G61 1QH, UK</li> <li><sup>4</sup> Livetec Systems Ltd, Building 52, Wrest Park, Silsoe, Bedford, MK45 4HS, UK</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Gampus, Edinburgh, Midlothian, EH25 9RG</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Gampus, Edinburgh, Midlothian, EH25 9RG</li> <li>Gampus, College of Medical</li> <li>Gampus, State and Cervical dislocation tools for despatching poultry</li> <li>Gampus, State and Cervical dislocation tools for despatching poultry</li> </ul>	al,
<ul> <li><sup>3</sup> Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medica Veterinary &amp; Life Sciences, University of Glasgow, G61 1QH, UK</li> <li><sup>4</sup> Livetec Systems Ltd, Building 52, Wrest Park, Silsoe, Bedford, MK45 4HS, UK</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> </ul>	al,
<ul> <li>Veterinary &amp; Life Sciences, University of Glasgow, G61 1QH, UK</li> <li><sup>4</sup> Livetec Systems Ltd, Building 52, Wrest Park, Silsoe, Bedford, MK45 4HS, UK</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Generation of the second secon</li></ul>	
<ul> <li><sup>4</sup> Livetec Systems Ltd, Building 52, Wrest Park, Silsoe, Bedford, MK45 4HS, UK</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> </ul>	
<ul> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>General Content of the second second</li></ul>	
<ul> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>General Content of the second second</li></ul>	
<ul> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools for despatching poultry</li> <li>Short title: Novel percussive and cervical dislocation tools fo</li></ul>	
15         16         17         18         19         20         21         22         23	
16         17         18         19         20         21         22         23	
17 18 19 20 21 22 23	
<ul> <li>18</li> <li>19</li> <li>20</li> <li>21</li> <li>22</li> <li>23</li> </ul>	
19 20 21 22 23	
20 21 22 23	
21 22 23	
22	
23	
24	
25	
26	
27	
28	
29	
30	
31	
32 *Corresponding author: Dr. Jossica Martin, The David (Diale) School of Materiaan Studies	
and The Roslin Institute Easter Rush Compus Edinburgh Midlethion EH25 OPC Tel:	>
+441316506062 Email: lessice Martin@ed.ac.uk	
36	

#### 37 Abstract

Four mechanical poultry killing devices; modified Armadillo<sup>®</sup> (MARM), modified Rabbit
 Zinger<sup>™</sup> (MZIN), modified pliers (MPLI) and a novel mechanical cervical dislocation
 gloved device (NMCD), were assessed for their killing potential in the cadavers of
 euthanised, of four bird type and age combinations: layer/adult, layer/pullet,
 broiler/slaughter-age, broiler/chick.

- A 4x4x4 factorial design (batch x device x bird type + age) was employed. Ten bird
  cadavers per bird type and age were tested with each of the four mechanical devices
  (N = 160 birds). All cadavers were examined post-mortem to establish the anatomical
  damage caused by each device.
- Three of the mechanical methods: NMCD, MARM and MZIN demonstrated killing
  potential, as well as consistency in their anatomical effects, with device success rates
  of over 50% indicating that the devices performed optimally more than half of the time.
  NMCD had the highest killing potential, with 100% of birds sustaining the required
  physical trauma to have caused rapid death.
- 52
  4. The MPLI was inconsistent, and only performed optimally for 27.5% of birds, despite
  53
  53
  54
  54
  55
  55
  56
  56
  56
- 57 5. This experiment provides important data on the killing potential of untried novel 58 percussive and mechanical cervical dislocation methods, informing future studies.
- 59

#### 60 Keywords

- 61 Killing; poultry; cervical dislocation; percussive; post-mortem; animal welfare.
- 62
- 63 Introduction

64 Worldwide, an estimated 9.1 billion birds may need to be killed on farm each year (DEFRA 2015) and the method with which these birds are killed therefore has relevance to poultry 65 66 welfare on a large scale. Poultry may need to be killed on-farm for multiple reasons (e.g. injury, 67 sickness and for stock management). Emergency killing on a large scale is often controlled by 68 whole-house or containerised gas methods (e.g. Lambooij et al., 1999; Gerritzen et al., 2004; 69 Gerritzen et al., 2009; McKeegan et al., 2011), but for the killing of smaller numbers of birds 70 on-farm, there are currently two main methods: (i) cervical dislocation, which is designed to 71 cause death by cerebral ischaemia and extensive damage to the spinal cord and brainstem 72 (Ommaya & Gennarelli 1974; Gregory & Wotton 1990; Erasmus et al., 2010a,b; Bader et al., 73 2014; Martin et al., 2016); and (ii) percussive devices designed to cause extensive brain 74 damage, resulting in brain death (Gregory & Wotton, 1990; HSA, 2004; Mason et al., 2009; 75 Erasmus et al., 2010a,b; Sparrey et al., 2014; Cors et al., 2015).

76

77 Cervical dislocation is one of the most prevalent methods for killing individual birds and is used in commercial and non-commercial contexts. It is perceived to be humane by users, is easy 78 79 to learn and perform, and does not require equipment (Mason et al., 2009; Sparrey et al., 80 2014; Martin, 2015; Martin et al., 2016). Both manual and mechanical cervical dislocation killing methods are designed to separate the skull from the vertebral column of the bird (ideally 81 82 C0–C1 vertebral dislocation), resulting in severing of the spinal cord and/or brainstem and the main blood vessels supplying the brain (Gregory & Wotton, 1990; Parent et al., 1992; Veras 83 84 et al., 2000; Cartner et al., 2007; Mason et al., 2009). It has been suggested that optimal application also produces a concussive effect on the bird due to trauma inflicted on the 85 brainstem through the action of stretching and twisting (Harrop et al., 2001; Shi & Pryor, 2002; 86 Pryor & Shi, 2006; Shi & Whitebone, 2006; Cartner et al., 2007; Erasmus et al., 2010a). 87 88 However, both methods of cervical dislocation have been the subject of welfare concern, as research in the last 40 years has raised questions about their humaneness and consistency 89 in poultry (Gregory& Wotton, 1986, 1990; Erasmus et al., 2010a), as well as other species 90 91 (Tidswell et al., 1987; Cartner et al., 2007). Some studies have indicated that animals,

92 including poultry, may be conscious for a significant period post-application of cervical dislocation (Gregory & Wotton, 1990; Erasmus et al., 2010a; Carbone et al., 2012) and it has 93 94 been noted that there is high variability in its application by different relevant groups (e.g. 95 poultry stock-workers, veterinarians, trained slaughtermen) (Mason et al., 2009; Sparrey et 96 al., 2014). Since January 2013 the use of manual cervical dislocation (MCD) as a killing 97 method for poultry on-farm has been heavily restricted through the new EU legislation, 98 Regulation (EC) no. 1099/2009 On the Protection of Animals at the Time of Killing (European Commission, 2009), following reported welfare concerns. In 2009, FAWC recommended 99 100 further research to explore current and novel methods for killing poultry in small numbers. 101 Several mechanical devices have been developed recently (e.g. CASH Poultry Killer, Turkey 102 Euthanasia Device) (Erasmus et al., 2010a; Erasmus et al., 2010b; HSA, 2004; Raj and 103 O'Callaghan, 2001), however, none have been enthusiastically adopted across the 104 commercial industry or by small poultry keepers.

105

Previous research has shown that post-mortem analysis is effective in inferring killing potential 106 107 and time to loss of consciousness and has been used across several species in determining 108 success rates of slaughter and on-farm killing method in livestock species while avoiding 109 ethical concerns associated with the application of new killing methods (e.g. Anil et al., 2002; 110 Grandin, 2010; Morzel et al., 2002; Bader et al., 2014). The successful application of cervical dislocation methods is determined by the animal having its neck dislocated and the spinal cord 111 112 severed (Bader et al., 2014; Carbone et al., 2012; Cartner et al., 2007; Erasmus et al., 2010a), while for concussive (head trauma) devices, there must be sufficient damage (e.g. skull 113 fractures, brain contusions, cerebral oedema, hemorrhaging and contra-coup damage (i.e. 114 damage to the brain on both sides: the side that received the initial impact (coup) and the side 115 116 opposite to the initial impact (countrecoup))) (Finnie et al., 2000; Finnie et al., 2002; Gregory et al., 2007; Gregory and Shaw, 2000). Such effects can be observed in cadavers following 117 the application of killing methods. Determining the success rate of a killing device is essential 118 119 to evaluating its overall efficacy, and the designing and prototyping of novel and modified

devices is the first stage of the development of a new humane device to despatch poultry onfarm. The aim of this study was to assess the potential killing performance of four novel or modified mechanical devices on both layer and broiler cadavers, through post-mortem analysis. The results can then inform the decision of whether the devices should be taken forward for further development and evaluation in live and conscious birds as potential new on-farm killing methods for chickens.

126

127

#### 128 Methods

#### 129 Subjects and husbandry

A total of 160 female layer-type (Hy-Line) and meat-type (Ross 308) chickens (*Gallus gallus domesticus*) were used in this study across four batches which were distributed equally across two types and ages (Table 1). Birds were sourced from commercial farms and transported to SRUC facilities in four batches of 40 birds per batch, with each batch containing all four bird type and age combinations. The birds were weighed and wing-tagged on arrival.

135

136 The birds were housed for one week prior to the experiment in order to allow them to acclimatise to the new environment and were housed in separate rooms per bird type and age 137 group to provide recommended environmental controls (Aviagen, 2009; Hy-Line, 2012). All 138 birds were kept in floor pens with wood-shavings litter at significantly lower than commercial 139 140 stocking density and with various environmental enrichments (e.g. suspended CDs, perches). The pens were constructed from wooden frames with wire-grid sides and roofs, allowing visual 141 and auditory contact with other birds within the same room. Broiler chicks and layer pullets 142 were housed in group pens (L 1.5 m x W 2.5 m x H 1.5 m). Broilers (slaughter-age) and layer 143 144 hens were kept in pairs (pen size: L 1.5 m x W 0.5 m x H 1.5 m). All birds had ad libitum access to appropriate food and water. All birds were inspected twice daily, and the minimum and 145 maximum temperatures were recorded each morning. 146

This experiment was performed under UK Home Office licence authority via Project and Personal licences and underwent review and approval (AUAE8-2012) by SRUC's ethical review body. All routine animal management procedures were adhered to by trained staff.

151

#### 152 Experimental Procedure

The experiment was designed around a 4 x 4 x 4 factorial design (batch x device x bird type + age). Ten birds per bird type (+ age) were tested with each of the four mechanical devices (N = 160 birds). Birds were tested in four one week batches, with birds being tested in blocks of ten per day in order to minimise any effect of operator fatigue (Sparrey *et al.*, 2014). A Graeco Latin square was used to balance batch, block, bird type (+ age) and device. Within this, 4 Latin squares (1 per batch) were used to balance block, test order in block and bird type (+age), with the test order in each block then repeated until all 10 birds were tested.

160

All birds were weighed and had schematic measurements of the head and neck were taken (Figure 2). Because it was inappropriate to evaluate un-tested killing methods on live birds, the birds were sequentially euthanised by an intravenous sodium pentobarbital injection (Euthatal, Merial Animal Health Ltd., Essex, UK) via the brachial vein immediately prior to device testing in order to minimise blood coagulation and morphological changes (Gordon *et al.*, 1988; Bell *et al.*, 1999).

167

168 Four mechanical poultry killing devices: modified Armadillo® (MARM), modified Rabbit ZingerTM (MZIN), modified pliers (MPLI) and a novel mechanical cervical dislocation gloved 169 device (NMCD) were assessed for their killing potential in cadaver birds (four bird type and 170 age combinations). All methods developed are discussed in detail in Martin (2015) and were 171 designed to comply with the current European legislation, EC1099/2009 (European Council, 172 2009). Briefly, the Armadillo<sup>®</sup> (Figure 1a) is a brain-stem penetrating device designed by a 173 veterinarian to dispatch game birds in the field (Sparrey et al., 2014; Martin, 2015). The device 174 175 consists of a scissor-type mechanism (approximately 17 cm in length); the bird's head is

176 placed into the 'cup' of the lower arm (beak facing downwards) and when ready to apply the 177 operator squeezes the handles together, which pushes the top arm (and the penetrating spike) 178 downwards into the back of the bird's skull, preferably through the foramen magnum therefore 179 severing the top of the spinal cord (or brain stem), and causing death by cerebral ischemia. 180 Presently there is no published scientific evidence on the efficacy of this device. Modifications 181 (with the permission of the inventor) consisted of replacing the lower arm of the device in order 182 to increase the upper (U) (33 mm to 37 mm) and lower (L) (19 mm to 27 mm) diameters of the openings of the metal cup based on pilot work demonstrating the need for a more space to 183 184 encompass chicken heads. Additional insertion cups were molded from 1mm thick plastic 185 funnels, in order to generate two adjustments (G1, G2) to fit the various sizes of birds' heads, based on bird type and age (G1: U=36 mm and L=23 mm (broiler, layer hen); G2: U=30 mm 186 and L=18 mm (layer pullets, broiler chicks)). The additional cups also had soft padding 187 188 (Waxman 4719095N <sup>1</sup>/<sub>2</sub> inch Self Stick Felt Pads, Waxman, Ohio, United States) added around the sides, which cushioned the lateral sides of the bird's head (over the eyes) as well as 189 creating an oval shape for the upper opening. 190

191

The Rabbit Zinger<sup>™</sup> (Pizzurro, 2009a,b) is a penetrating captive-bolt device originally 192 193 designed to kill rabbits (Figure 1b). It uses the stored energy in rubber tubes to drive a 194 penetrating bolt into the animal's head, causing death by extensive irreversible brain damage (DEFRA, 2014; Martin, 2015). The device was modified with permission of the original 195 196 designer in order to adapt it to the new target species (i.e. poultry), however the original function and bolt mechanism of the device was retained. The blue Power Tubes<sup>™</sup> (Pizzurro, 197 2009a) were used, which require 177 N to pull the bolt into the cocked position (Sparrey et al., 198 2014; Martin et al., 2016) and when fired the bolt (0.6 mm diameter) delivered approximately 199 11.87 J of kinetic energy. The modifications have been described previously (Martin, 2015; 200 Martin et al., 2016), but consisted of three aluminium appendages added to the base of the 201 device in order to provide a method of gently restraining the bird's head: two rested either side 202 203 of the bird's head (over the ears, orauricular feathers) and the third ran down the front of the

bird's face between the eyes and over the nostrils and beak. Additional leather washers
(Pizzurro, 2009a,b) were added to the bolt, in order to reduce the penetration depth from 3.5
to 2.5 cm. The MZIN device was also weighted at the bottom in order to counteract the topheaviness of the device when cocked.

208

209 'Semark' pliers (also known as the 'Humane Bird Dispatcher') weigh approximately 200 g and 210 have an overall length of 180 mm. When the blades of the device are fully open the maximum distance between the upper and lower teeth is 36 mm. When the blades are fully closed there 211 212 is a slight gap between the blades (<1 mm). The pliers were modified (MPLI) in an attempt to 213 reduce reported crushing injury (DEFRA 2014) by adapting the shape and width of the blades in order to create a narrower, curved concave edge rather than a straight edge (Martin, 2015). 214 The edges of the blades remained blunt in order to reduce the risk of skin tearing and thus 215 216 blood loss during application of the method. It was hypothesised that by narrowing the edge of the blade it would reduce the risk of crushing and would instead increase the likelihood of 217 218 dislocation, as the narrower blade would more easily slip between two cervical vertebra when 219 force was applied. The blades were widened gradually to increase the size of the blade (over 220 3 mm) and therefore generate a dislocation (i.e. gap between the two vertebra), by pushing 221 the vertebrae apart.

222

The NMCD device (Figure 1d) was designed to create a mechanical method for cervical 223 224 dislocation of poultry which mirrored the technique of the manual method (described in Martin, 2015; Martin et al., 2016). The device consisted of a thin supportive glove (SHOWA 370 225 Multipurpose Stable Glove<sup>™</sup>, UK) designed to support the wrist and hand (and hypothesised 226 to reduce strain injury in the operator) and a moveable metal insert. The metal insert consisted 227 228 of two metal finger supports that were designed to fit around the bird's head to create a secure 229 grip, and to move independently from side-to-side in order to allow adjustment for different sizes of birds (Figure 1d). The rounded shape of the metal fingers was designed to aid the 230 231 twisting motion (performed during manual cervical dislocation (Sparrey et al., 2014; Martin et *al.*, 2016)) required to dislocate the bird's neck by enhancing the 'rolling action' of the hand.
The blunt edge between the two metal fingers (protruding < 1 mm from the fleshy area of skin</li>
between the index and middle fingers) provided a hard edge to force between the back of the
bird's head and the top of the neck, designed to focalise the force into the desired area (i.e. a
dislocation at C0–C1) when the method was applied.

- 237
- 238

After device application, cadavers were immediately examined post-mortem in order to 239 240 establish as accurately as possible the anatomical damage caused by the device. Specific 241 post-mortem measures were recorded for each killing device as their target anatomical areas were different. For all killing devices, binary measures (yes/no) were recorded for skin broken, 242 external blood loss and subcutaneous hematoma and the total number of attempts were 243 244 recorded (e.g. multiple pulls for NMCD or miss-fire of MZIN). For the MZIN and MARM, seven 245 specific measures were recorded: binary measures of damage to the skull, specific brain 246 regions (left forebrain, right forebrain, cerebellum, midbrain and brainstem); and the presence 247 of an internal brain cavity hematoma. For killing devices which caused trauma to the neck of 248 the bird (NMCD and MPLI), seven specific post-mortem measures were assessed including four binary measures (dislocation of the neck, vertebra damage (e.g. intra-vertebra 249 250 dislocation/break), damage to neck muscle, crushing injury to the trachea or oesophagus and whether the spinal cord was severed). The level of cervical dislocation was also recorded (e.g. 251 252 between C0-C1, C1-C2, C2-C3, etc.). The number of carotid arteries severed was also 253 recorded as zero, one or both.

254

#### 255 Derived kill potential and device success

From the post-mortem evaluations two further binary (yes/no) measures were derived: kill potential and device success. Kill potential was defined as the cadaver exhibiting sufficient damage to any part of the anatomy which would have resulted in death (if the bird had been alive at testing) following one attempt. For example, this was confirmed dislocation of the neck and severing of the spinal cord for NMCD and MPLI (Bader et al., 2014; Erasmus et al., 2010a;
Gregory and Wotton, 1990); and diffuse brain damage for the MARM and MZIN (Finnie *et al.*,
2000; Finnie *et al.*, 2002; Limon *et al.*, 2010) after one attempt.

263

Device success was defined as when the device caused the desired anatomical damage, dictated by its hypothesised design, as well as producing sufficient damage which would have resulted in death (if the bird had been alive at testing) and based on scientific literature would be most likely to minimise time to unconsciousness post device application. Device success criteria were device specific and are described in Table 2.

269

#### 270 Statistical Analysis

271 All data were summarised in Microsoft Excel (2010) spread sheets and analysed using Genstat (14th Edition). Statistical significance was based on F statistics and P<0.05 272 significance level. Summary graphs and statistics were produced at bird and treatment level. 273 Generalised Linear Mixed Models (GLMM) (binomial distribution) were used to compare 274 275 performance across the four killing devices in terms of kill potential and device success, while 276 incorporating bird type, age, and block as fixed effects and bird weight head measurements as co-variates. Batch was included as a random effect. Detailed comparisons of device 277 performance were achieved by sub setting the data twice: initially to remove unsuccessfully 278 "killed" birds (i.e. kill potential "no") in order to prevent data skewing; and then into two groups 279 280 dependent on trauma area: 1) neck trauma (NMCD and MPLI); and (2) head trauma (MZIN and MARM), in order to allow logical comparison between killing treatments which damaged 281 the neck or the head. Statistical comparisons on anatomical measures were conducted via 282 GLMMs (Poisson distribution and binomial distribution) or Linear Mixed Models (LLM) (normal 283 284 distribution) dependent on the data distributions for each variable. Data transformations were attempted when necessary via Logarithm function. All models included batch number as 285 random effects. All fixed effects were treated as factors and classed as categorical 286 287 classifications and all interactions between factors were included in maximal models.

288

#### 289 Results

290 A total of 36 birds were not successfully "killed" on the first attempt (NMCD = 0/40 birds; MPLI 291 = 15/40 birds; MARM = 15/40 birds; and MZIN = 6/40 birds). Device had an effect on kill 292 potential (F<sub>(3.144)</sub>=2.88, P=0.038), with NMCD having the highest kill potential, with 100% of 293 birds sustaining the required physical trauma to have caused death (Figure 3). The MARM 294 and MPLI had the lowest kill potential, both achieving 62.5%. Bird age was the only other factor to affect kill potential ( $F_{(1,144)}$ =5.15, P=0.025), with younger birds being more likely to 295 296 sustain the required physiological trauma to have resulted in death (mean =  $0.87 \pm 0.04$ ), 297 compared to older birds (mean =  $0.68 \pm 0.05$ ). All other factors (bird weight, type and head 298 measures) and their interactions had no effect on kill potential.

299

Device success was affected by killing device ( $F_{(3,144)}=7.00$ , P<0.001), with NMCD shown to be most likely to perform in the desired way and producing optimal damage (Figure 3). Like kill potential, bird age affected device success ( $F_{(1,144)}=5.03$ , P=0.026), with younger birds (mean = 0.69 ± 0.05) being more likely to sustain optimal anatomical damage compared to older birds (mean = 0.53 ± 0.06). All other factors and their interactions had no effect on device success.

306

#### 307 Percussive methods

308 For successfully killed birds (MARM = 25/40 birds; and MZIN = 34/40 birds), the percentage of birds for which the relevant head trauma post mortem factor was present, according to 309 killing method is shown in Table 3. Killing device had no effect on the majority of post-mortem 310 311 measures, apart from damage to left forebrain, mid brain, and brain stem. The MZIN was 312 significantly more likely to cause trauma to the left forebrain and the mid brain compared to the MARM, however, the opposite was seen for the brain stem, with very few MZIN birds 313 sustaining damage compared to the MARM. No other factor or interaction affected external 314 315 bleeding, skin tearing, subcutaneous hematoma, or whether or not the skull was damaged.

Bird type, bird age, bird weight and their interactions with killing method had no effect on damage to any region of the brain.

318

#### 319 Cervical dislocation methods

320 For successfully killed birds (MPLI = 25/40 birds; NMCD = 40/40 birds), the percentage of 321 birds for which the relevant neck trauma post mortem factor was present, according to killing 322 method, is shown in Table 4. Numerically, MPLI was more likely to tear the skin, cause external 323 bleeding, vertebral damage, trachea damage, and oesophagus damage compared to NMCD, 324 but the differences were not significant. NMCD was more likely to cause cervical dislocation, 325 as well as severing one or more carotid arteries compared to MPLI (Figure 4). However, the location of the dislocation (e.g. C0-C1, C1-C2, etc.) was not significantly affected by killing 326 method ( $F_{3.74}$ =2.34, P=0.076), although there was a tendency (P < 0.10), for NMCD to be 327 328 more likely to cause a higher level dislocation compared to MPLI (Figure 5).

329

Whether or not cervical dislocation (no = 0; yes = 1) occurred was significantly affected by bird 330 331 type ( $F_{1.74}$ =5.98, P=0.014) and bird age ( $F_{1.74}$ =6.39, P=0.011), with dislocations more likely 332 to occur in broilers (mean =  $0.95 \pm 0.05$ ) rather than layers (mean =  $0.55 \pm 0.11$ ), and younger birds (mean =  $0.90 \pm 0.07$ ) compared to older birds (mean =  $0.60 \pm 0.11$ ). The diameter of the 333 birds' necks (N1) (F<sub>1.74</sub>=4.00, P=0.050) also had an effect with unsuccessful dislocations 334 associated with larger neck diameters (17.1±1.09 mm) compared to successful dislocations 335 336  $(14.9\pm0.51 \text{ mm})$ . Bird type had an effect on the likelihood of vertebral damage (no = 0; yes = 1), with layers (mean =  $0.75 \pm 0.10$ ) more likely to sustain damage than broilers (mean = 0.35337 ± 0.11). No other factors or interactions, apart from killing method (reported above) had an 338 339 effect on vertebral damage.

340

Bird type, bird age, and bird weight and their interactions with killing device had no effect on
skin tearing, external bleeding, subcutaneous, hematoma, trachea damage, oesophagus
damage, number of carotid arteries severed, dislocation level, and dislocation level. The neck

diameter of the birds (N1) had a tendency to affect the number of carotid arteries severed ( $F_{1,74}$ =3.31, *P*=0.074), with a significant negative correlation (*r* = -0.382, *P* = 0.047).

346

#### 347 Discussion

348 The results of this experiment provide important data to allow evaluation of the killing potential 349 of four untried novel percussive and mechanical cervical dislocation methods for chickens. 350 The devices had been designed and prototyped with the aim to cause rapid loss of consciousness and brain death in order to be effective and humane. The NMCD device was 351 352 shown to have the highest killing potential (100%), however, all devices achieved a killing 353 potential of over 60%. NMCD was also shown to have the highest device success (90%), demonstrating its consistency in achieving optimal damage to the cadavers, irrespective of 354 bird type. Device success was always lower than the killing potential for each method because 355 356 it was a more specific measure. The difference between killing potential and devices success was approximately 10% for NMCD, MZIN and MARM, demonstrating that these methods were 357 not always performing optimally, which could have welfare implications. For NMCD, the 358 primary reason for this difference was the number of carotid arteries severed, as on occasion 359 360 only one was severed, and some birds exhibited a lower dislocation level than C0-C1. In the case of MZIN, the few failures in device success were due to only one region of the brain being 361 damaged or only minor damage to all regions (e.g. internal brain cavity bleeding and bruising). 362 Failures in device success with the MARM were primarily due to the spike not penetrating to 363 364 an adequate depth to cause complete severing of the brain stem, as well as some issues with the ability to aim the device easily, and the spike not penetrating the brain stem, but instead 365 the cerebellum. In terms of brain trauma, this could reduce the chance of neurogenic shock 366 and elongate the time to loss of consciousness and brain death (Alexander, 1995; Dumont et 367 368 al., 2001; Freeman and Wright, 1953; White and Krause, 1993), but it did not appear to affect 369 the inferred kill potential (i.e. the damage would still be fatal).

The MARM and MPLI had the lowest kill potential at 62.5%, however the MPLI had significantly lower device success (27.5%) than its killing potential. This was primarily because more than 50% of birds showed vertebral damage, failure of dislocation and trachea damage, which was representative of severe crushing injury and inference of causing death by asphyxiation, which is a serious welfare concern (Erasmus *et al.*, 2010a; Gregory and Wotton, 1990; Salim *et al.*, 2006; Sharma *et al.*, 2005).

377

378 Post-mortem measures for the neck trauma methods highlighted that the MPLI caused 379 numerically more instances (though not significant) of cause skin tears and external bleeding, 380 which could be considered a practical issue in a commercial environment due to biosecurity, human health and safety as well as being visually un-appealing (Gerritzen and Raj, 2009; 381 Halvorson and Hueston, 2006; Kingsten et al., 2005). The MPLI, designed to dislocate the 382 383 cervical vertebrae, only caused dislocation 45% of the time and caused crushing injury to the trachea as well as to the oesophagus. The injuries sustained, as well as the pressure applied 384 by the blades, would still be fatal, but would not necessarily cause death by cerebral ischemia, 385 386 which is the intended outcome (Veras et al., 2000; Harrop et al., 2001; Bader et al., 2014). 387 The primary concern with MPLI was that, despite the modifications, it was not performing in the desired way, indicating that it was not a reliable method. 388

389

Both the MARM and MZIN always caused penetration of the skin and damage to the skull and 390 391 the majority of birds bled into the external environment. There were significant differences in the areas of the brain damaged by the two devices, but they were designed to perform 392 differently. With the MZIN, more than 60% of all birds received damage to the main areas of 393 the brain (excluding the brain stem), demonstrating diffuse damage which the device is 394 designed to cause in order to cause concussion and brain death (Alexander, 1995; Finnie et 395 al., 2000; Oppenheimer, 1968). The MZIN showed higher killing potential than the unmodified 396 Rabbit Zinger<sup>™</sup>, which had previously been reported to have a kill success rate of 50% in 397 398 poultry (DEFRA, 2014). The MARM caused focalised damage to the brain stem and 399 cerebellum, highlighting that the modifications to the MARM had adequately adapted its design 400 to more adequately fit poultry. Such damage to the brain stem theoretically would result in 401 fatal functional impairment (e.g. puntilla method as described in Limon et al., 2009; Limon et 402 al., 2010) (HSA, 2004; Morzel et al., 2002; Widjicks, 1995). The un-modified Armadillo® was 403 tested previously (DEFRA, 2014), and was found to have a low kill success of 46%, therefore 404 the higher kill potential could be attributed to the modifications or that the killing potential was 405 tested on cadavers, which are easier to handle, improving application of the method. The 406 increase in success in the MZIN could be attributed to the same reasons.

407

408 Other bird factors were shown to impact some post-mortem measures (e.g. dislocation level, 409 vertebral damage), kill potential and device success, demonstrating inconsistency dependent on the target species, although their impact was more pronounced with the cervical dislocation 410 411 methods than the head trauma methods. Bird age affected both killing potential and device 412 success, in both cases revealing that it was easier to cause physiological trauma to younger birds and therefore easier to achieve a reliable kill. Young birds are less physiologically 413 414 mature, and therefore bones and cartilage are less calcified and re-inforced, as well as 415 connective tissue being less fibrous, making dislocation and damage to the skull easier to achieve (Comi et al., 2009; Sharma et al., 2005). However, in terms of neck muscle and arterial 416 tissue, aging can have a detrimental effect, with reduced elasticity in arterial walls and skeletal 417 muscle, reducing stretching potential, therefore carotid arteries and neck muscle are more 418 419 likely to tear when under strain (Benetos et al., 1993; Nair, 2005). However this needs to be considered in context of the size of the birds; smaller birds have less stretch potential than 420 larger birds, therefore despite the increased elasticity, the magnitude of the stretch required 421 to dislocate and tear should counteract this effect. In general, cervical dislocation was easier 422 423 in broilers and younger birds, although these factors are confounded, as by definition broilers 424 at both ages tested were immature compared to layer strains. The diameter of the neck also affected dislocation potential, with smaller necks (younger birds) being easier to dislocate than 425 larger necks (older birds). When considering vertebral damage, layers were more likely to 426

427 receive damage, but again bird type was confounded with age, with laying hens being older 428 than any other bird group. The increased likelihood of vertebral damage could also be 429 attributed to brittle bones in the laying hens (Whitehead and Fleming, 2000). All other external 430 factors had no impact on the post-mortem measures associated with brain trauma methods, 431 indicating that these methods are less susceptible to inconsistency as when applied to various 432 types, size and age of birds. However, this has to be taken within the context that both of the 433 brain trauma methods: MZIN and MARM had killing potentials of 84.2% and 62.5% respectively, both of which highlight issues with reliability. 434

435

436 This study provides a general assessment of prototyped novel and modified devices for killing poultry on-farm, and the results demonstrate their killing potential. Three of the mechanical 437 methods: NMCD, MARM and MZIN demonstrated killing potential, as well as consistency in 438 439 their physical effects. Device success rates of over 50% demonstrated that more than half the time the devices performed optimally. In future studies, more detailed assessment of post-440 mortem evaluations would be desirable, for example, skull damage location and size of 441 442 dislocation (i.e. measurement of gap between two dislocated vertebrae), in order to further 443 establish the effects on anatomy and more accurately infer time to unconsciousness and brain death in live birds. The MPLI was inconsistent, and had a low device success of 27.5%, despite 444 matching killing potential with the MARM. The abundant evidence of crushing injury in >50% 445 of birds was also a major concern, especially as the new European legislation on the 446 447 Protection of Animals at the Time of Killing bans by their omission, the use of any method which demonstrates death by crushing to the neck (European Council, 2009). Thus, MPLI are 448 not recommended as a humane on-farm killing device for chickens. The performance of the 449 remaining three devices (NMCD, MZIN, MPLI) will be further assessed in live birds in order to 450 451 establish their potential to provide a new humane method for despatching poultry on-farm.

452

#### 453 Acknowledgements

454 This research was funded by the Humane Slaughter Association (HSA), UK via their 455 Centenary Research Training Scholarship (RTS) awarded to Jessica Martin in 2011.

456

#### 457 **References**

- Alexander, M.P., (1995). Mild traumatic brain injury: pathophysiological, natural history, and
   clinical management. *Neurology* **45**: 1253-1260.
- Anil, S., Anil, S.L., Deen, J., (2002). Challenges in pain perception of domestic animals.
   *Journal of American Veterinary Medical Association*. **220**: 313-319.
- Bader, S., Meyer-Kûhling, B., Günther, R., Breithaupt, A., Rautenschlein, S., Gruber, A.D.,
  2014. Anatomical and histologic pathology induced by cervical dislocation following
  blunt head trauma for on-farm euthanasia of poultry. *Journal of Applied Poultry Research.* 23: 546-556.
- Bell, L. S., Skinner, M. F., and Jones, S. J., (1996). The speed of post mortem change to the
  human skeleton and its taphonomic significance. *Forensic Science International*, 82
  (2): 129-140
- Benetos, A., Laurent, S., Hoeks, A.P., Boutouyrie, P.H., Safar, M.E., (1993). Arterial
  alterations with aging and high blood pressure. A noninvasive study of carotid and
  femoral arteries. *Arteriosclerosis, Thrombosis, and Vascular Biology.* 13: 90-97.
- 472 Carbone, L.G., Carbone, E.T., Yi, E.M., Bauer, D.B., Lindstrom, K.A., Parker, J.M., Austin,
- J.A., Seo, Y., Gandhi, A.D., Wilkerson, J.D., (2012). Assessing cervical dislocation as
  a humane euthanasia method for mice. *Journal of the American Association for Laboratory Animal Science*, **51**: 352-356.
- 476 Cartner, S.C., Barlow, S.C., Ness, T.J., (2007). Loss of Cortical Function in Mice After
  477 Decapitation, Cervical Dislocation, Potassium Chloride Injection, and CO2 Inhalation.
  478 *Comparative Medicine*. **57**: 570-573.
- Comi, A.M., Trescher, W.H., Abi-Raad, R., Johnston, M.V., Wilson, M.A., (2009). Impact of
  age and strain on ischemic brain injury and seizures after carotid artery ligation in
  immature mice. *International Journal of Developmental Neuroscience*, **27**: 271-277.

482 DEFRA, (2015). DEFRA United Kingdom Poultry and Poultry Meat Statistics - March 2015,

- 483 https://www.gov.uk/government/uploads/system/uploads/attachment\_data/file/423075/
  484 poultry-statsnotice-23apr15.pdf
- DEFRA, (2014). DEFRA (MH0145) Welfare costs and benefits of exizting and novel on-farm
  culling methods for poultry. In: McKeegan, D.E.F., Martin, J.E., Sandilands, V.,
  Sandercock, D.A., Sparrey, J.M., Sparks, N.H.C. (Eds.), DEFRA Publications, UK.
- 488 Dumont, R.J., Okonkwo, D.O., Verma, S., Hurlbert, R.J., Boulos, P.T., Ellegala, D.B., Dumont,
  489 A.S., (2001). Acute Spinal Cord Injury, Part I: Pathophysiologic Mechanisms. *Clinical*490 *Neuropharmacology*. 24: 254-264.
- Erasmus, M.A., Lawlis, P., Duncan, I.J.H., Widowski, T.M., (2010a). Using time insensibility
  and estimated time of death to evaluate a nonpenetrating captive bolt, cervical
  dislocation, and blunt trauma for on-farm killing of turkeys. *Poultry Science*. 89: 13451354.
- Erasmus, M.A., Turner, P.V., Nykamp, S.G., Widowski, T.M., (2010b). Brain and skull lesions
  resulting from use of percussive bolt, cervical dislocation by stretching, cervical
  dislocation by crushing and blunt trauma in turkeys. *Vetinary Record.* 167: 850-858.
- European Council, (2009). European Council Regulation (EC) 1099/2009 of 24 September 498 499 2009 on the Protection of Animals at the Time Killing. of In. ec.europa.eu/food/animal/welfare/slaughter/regulation\_1099\_2009\_en.pdf. Accessed 500 28/09/2011. 501
- FAWC, (2009). FAWC Report on Farm Animal Welfare in Great Britain: Past, Present and
  Future. In. FAWC advice to government Animal welfare.
  http://www.fao.org/fileadmin/user\_upload/animalwelfare/ppf-report091012.pdf
  Accessed 03/02/2012.
- Finnie, J.W., Blumbergs, P.C., Manavis, J., Summersides, G.E., Davies, R.A., (2000).
  Evaluation of brain damage resulting from penetrating and non-penetrating captive bolt
  stunning using lambs. *Australian Veterinary Journal*. **78**: 775-778.

- 509 Finnie, J.W., Manavis, J., Blumbergs, P.C., Summersides, G.E., (2002). Brain damage in 510 sheep from penetrating captive bolt stunning. *Australian Veterinary Journal*. **80**: 67-69.
- 511 Freeman, L.W., and Wright, T.W., (1953). Experimental Observations of Concussion and 512 Contusions of the Spinal Cord. *Annals of Surgery*. **137**: 433-443.
- Gerritzen, M.A., Lambooij, B., Reimert, H., Stegeman, A., Spruijt, B., (2004). On-farm
  euthanasia of broiler chickens: effects of different gas mixtures on behavior and brain
  activity. *Poultry Science*, 83: 1294-1301.
- 516 Gerritzen, M.A., Raj, A.B.M., (2009). Animal welfare and killing for disease control. In:
  517 Smulders, F.J.M. (Ed.), Welfare of Production Animals: Assessment and Management
- of Risks, Wageningen Acad Publ, pp. 191-203.
- Gordon, I., Shapiro, H.A., and Berson, S.D., (1988). Forensic Medicine (2nd edn.) Churchill
  Livingstone, Edinburgh and London, pp. 1–62
- 521 Grandin, T., (2010). Auditing animal welfare at slaughter plants. *Meat Science*. **86**: 56-65.
- 522 Gregory, N.G., Lee, C.J., Widdicombe, J.P., (2007). Depth of concussion in cattle shot by 523 penetrating captive bolt. *Meat Science*. **77**: 499-503.
- 524 Gregory, N.G., Shaw, F., (2000). Penetrating Captive Bolt Stunning and Exsanguination of 525 Cattle in Abattoirs. *Applied Animal Behaviour Science*. **3**: 215-230.
- 526 Gregory, N.G., Wotton, S.B., (1986). Effect of slaughter on the spantaneous and evoked 527 activity of the brain. Brit. *Poultry Science*. **27**: 195-205.
- Gregory, N.G., Wotton, S.B., (1990). Comparison of neck dislocation and percussion of the
  head on visual evoked responses in the chicken's brain. *Vetinary Record*. **126**: 570572.
- Halvorson, D.A., Hueston, W.D., (2006). The Development of an Exposure Risk Index as a
  Rational Guide for Biosecurity Programs. *Avian Diseases*. **50**: 516-519.
- Harrop, J., Sharan, A., Vaccaro, A.R., Przybylski, G.J., (2001). The Cause of Neurologic
  Deterioration After Acute Cervical Spinal Cord Injury. *Spine*. 26: 340-346.
- HSA, 2004. Practical Slaughter of Poultry: A Guide for the Small Producer. Humane Slaughter
  Association. United Kingdom.

- Kingsten, S.K., Dussault, C.A., Zaidlicz, R.S., Faltas, N.H., Geib, M.E., Taylor, S., Holt, T.,
  Porter-Spalding, B.A., (2005). Evaluation of the two methods of mass euthanasia of
  poultry in disease outbreaks. *Journal of American Veterinary Medical Association*. 227:
  730-738.
- Lambooij, E., Gerritzen, M.A., Engel, B., Hillebrand, S.J.W., Lankhaar, J., Pieterse, C., (1999).
   Behavioural responses during exposure of broiler chickens to different gas mixtures.
- 543 Applied Animal Behaviour Science, **62**: 255-265.
- Limon, G., Guitian, J., Gregory, N.G., (2009). A note on the slaughter of llamas in Bolivia by
  the puntilla method. *Meat Science*. 82: 405-406.
- Limon, G., Guitian, J., Gregory, N.G., (2010). An evaluation of the humaneness of puntilla in
  cattle. *Meat Science*. 84: 352-355.
- Martin, J. E., (2015). Humane mechanical methods to kill poultry on-farm. *Ph.D. Thesis*,
  University of Glasgow.
- 550 Martin, J. E., McKeegan, D. E. F., Sparrey, J., and Sandilands, V., (2016). Comparison of
- 551 novel mechanical cervical dislocation and a modified captive bolt for on-farm killing of
- poultry on behavioural reflex responses and anatomical pathology. *Animal Welfare*, 25
  (2): 227-241
- Mason, C., Spence, J., Bilbe, L., Hughes, T., Kirkwood, J., (2009). Methods for dispatching
  backyard poultry. *Veterinary Record* 164: 220.
- McKeegan, D.E.F., Sparks, N.H.C., Sandilands, V., Demmers, T.G.M., Boulcott, P., Wathes,
   C.M., (2011). Phyioslogical responses of laying hens during whole house killing with
   carbon dioxide. *British Poultry Science*, **52**: 645-657.
- Morzel, M., Sohier, D., Van de Vis, H., (2002). Evaluation of slaughtering methods for turbot
  with respect to animal welfare and flesh quality. *Journal of the Science of Food and Agriculture* 82: 19-28.
- 562 Nair, K.S., (2005). Aging muscle. *The American Journal of Clinical Nutrition* **81**: 953-963.
- 563 Ommaya, A.K., Gennarelli, T.A., (1974). Cerebral concussion and traumatic 564 unconsciousness. *Brain* **97**: 633-654.

- 565 Oppenheimer, D.R., (1968). Microscopic lesions in the brain followings head injury. *Journal* 566 *Neurology, Neurosurgery & Psychiatry* **31**: 299-306.
- 567 Parent, A., Harkey, L.H., Touchstone, D.A., Smit, E.E., Smith, R.R., (1992). Lateral Cervical
  568 Spine Dislocation and Vertebral Artery Injury. *Neurosurgery* **31**: 501-509.
- 569 Pizzurro, S., (2009a). About us expectation of order fulfilment. United States.
- 570 Pizzurro, S., (2009b). Zinger Stun Guns<sup>™</sup> The Rabbit Zinger<sup>™</sup>, (TRZ001). United States.
- 571 Pryor, J.D., Shi, R., (2006). Electrophysiological changes in isolated spinal cord white matter
  572 in response to oxygen deprivation. *Spinal Cord* 4: 653-661.
- 573 Raj, A.B.M., O'Callaghan, M., (2001). Evaluation of a pneumatically operated captive bolt for
  574 stunning/killing broiler chickens. *British Poultry Science* 42: 295-299.
- Salim, A., Martin, M., Sangthong, B., Brown, C., Rhee, P., Demetriades, D., (2006). Nearhanging injuries: A 10-year experience. *Injury, International Journal of the Care of the Injured* 37: 435-439.
- Sharma, B.R., Singh, V.P., Harish, D., (2005). Neck Structure Injuries in Hanging Comparing
   Retrospective and Prospective Studies. *Medical, Science and Law* 45: 321-330.
- Shi, R., Pryor, J.D., (2002). Pathological Changes of Isolated Spinal Cord Axons in Respnse
  to Mechanical Stretch. *Neuroscience* **110**: 765-777.
- Shi, R., Whitebone, J., (2006). Conduction Deficits and Membrane Disruption of Spinal Cord
  Axons as a Fcuntion of Magnitude and Rate of Strain. *Journal of Neurophysiology* 95:
  3384-3390.
- Sparrey, J.M., Sandercock, D.M., Sparks, N.H.C., Sandilands, V., (2014). Current and novel
  methods for killing poultry individually on-farm. *World Poultry Science Journal* **70**(4):
  737-758.
- Tidswell, S.J., Blackmore, D.K., Newhook, J.C., (1987). Slaughter methods:
  Electroencephalographs (EEG) studies on spinal cord section, decaptitation and gross
  trauma of the brain in lambs. *New Zealand Veterinary Journal* 35: 46-49.

591	Veras, L., Pedraza-Gutiérrez, S., Castellanos, J., Capellades, J., Casamitjana, J., Rovira-
592	Cañellas, A., (2000). Vertebral Artery Occlusiion After Acute Cervical Spine Trauma.
593	Spine <b>25</b> : 1171-1177.

- White, B.C., Krause, G.S., (1993). Brain injury and repair mechanisms: the potential for
  pharmacologic therapy in closed-head trauma. *Annals of Emergency Medicine* 22:
  970-979.
- 597 Whitehead, C.C., Fleming, R.H., (2000). Osteoporosis in Cage Layers. *Poultry Science* 79:
  598 1033-1041.
- 599 Widjicks, E.F.M., (1995). Determining brain death in adults. *Neurology* **45**: 1003-1011.

Table 1: Accommodation and bird details for each bird type and age group.

Bird group	Ν	Mean bird age at killing (days)	Mean bird weight at killing (kg)	Housed stocking density (kg/m²)
Layer pullets	40	73.5 ± 0.2	0.8 ± 0.1	2.3
Layer hens	40	487.9 ± 0.9	1.8 ± 0.1	4.8
Broiler chicks	40	$22.4 \pm 0.1$	0.7 ± 0.2	1.9
Broiler (slaughter age)	40	37.1 ± 0.6	$1.9 \pm 0.7$	5.1

604 Table 2: Device success parameters for each killing device.

Device	Device success criteria
MARM	Spike penetrates through foramen magnum of the skull
	Severing of brain stem
MZIN	Skull is penetrated and damaged
	<ul> <li>Severe damage to a minimum of one area of the brain</li> </ul>
MPLI	Complete cervical dislocation at C0-C1
	<ul> <li>Severing of the top of the spinal cord (i.e. brain stem)</li> </ul>
	<ul> <li>Severing of both carotid arteries</li> </ul>
	No breakage to the skin
	<ul> <li>No crushing injury to the trachea or oesophagus</li> </ul>
NMCD	Complete cervical dislocation at C0-C1
	<ul> <li>Severing of the top of the spinal cord (i.e. brain stem)</li> </ul>
	<ul> <li>Severing of both carotid arteries</li> </ul>
	No breakage to the skin

Table 3: Percentage of birds killed successfully for which the relevant head trauma post

	Percentage of birds			
Post mortem measure	MZIN	MARM	F statistic	P value
Skin broken	100.0	100.0	0.03	0.993
External bleeding	96.7	88.0	1.44	0.264
Subcutaneous hematoma	100.0	92.0	1.44	0.234
Skull damage	100.0	100.0	0.06	0.982
Left forebrain damage	62.5	0.0	5.81	0.029
Right forebrain damage	65.6	0.0	4.70	0.994
Cerebellum damage	65.6	64.0	0.00	0.998
Midbrain damage	84.4	0.0	5.80	<u>0.013</u>
Brain stem damage	31.3	92.0	5.10	<u>0.034</u>

608 mortem factor was present, according to killing method. Significant P values are underlined.

609

Table 4: Percentage of birds killed successfully for which the relevant neck trauma post

Deat martam managem	Percentage of birds		<b>F</b> atatiatia	
Post mortem measure	NMCD	MPLI	- F statistic	P value
Skin broken	7.5	20.0	0.32	0.570
External bleeding	2.5	7.5	0.06	0.805
Subcutaneous hematoma	100.0	72.5	0.00	0.994
Cervical dislocation	100.0	45.0	11.86	<0.001
Vertebral damage	5.0	55.0	3.26	0.071
≥1 carotid artery severed	95.0	15.0	6.34	<u>0.012</u>
Trachea damage	0.0	52.5	3.41	0.059
Oesophagus damage	0.0	12.5	0.13	0.870
Spinal cord severed	100.0	67.5	0.00	0.998

612 mortem factor was present, according to killing method. Significant P values are underlined.

### 613

a) Armadillo® (MARM)



b) Rabbit Zinger™ (MZIN)



c) 'Semark' pliers (MPLI)



d) Novel mechanical cervical dislocation gloved device (NMCD)



616

- 617 Figure 1: Photographs of tested devices: a) Armadillo<sup>®</sup>, b) Rabbit Zinger<sup>™</sup>, c) 'Semark'
- 618 pliers, and d) the Novel mechanical cervical dislocation gloved device.





Figure 2: Schematic showing head and neck measures: A = width of head; B = lower

- 622 mandible to top of skull; D = width of base of beak; E = base of skull to front of beak; F =
- width of beak at central nostril level; G = depth of beak; and N1 = width of neck.





Figure 3: Summary of kill potential and device success rates (%) across the four killing

627 devices. No common lettering indicates that there is a significant difference between the

- 628 groups.
- 629



631

Figure 4: Percentage of birds by the number of carotid arteries severed dependent on killing

633 method. No common lettering indicates that there is a significant difference between the

634 groups.



637 Figure 5: Distribution of birds by the various dislocation levels in relation to killing method.