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

Published In:
British Poultry Science

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

Jessica E Martin[1,2,3*], Dorothy E F McKeegan[3], Julian Sparrey[4] and Victoria Sandilands[1]

[1] Animal Behaviour and Welfare, Animal and Veterinary Science Research Group, SRUC, Roslin Institute Building, Easter Bush, Edinburgh, EH25 9RG, UK

[2] The Royal (Dick) School of Veterinary Studies and The Roslin Institute, Easter Bush Campus, Edinburgh, Midlothian, EH25 9RG

[3] Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary & Life Sciences, University of Glasgow, G61 1QH, UK

[4] Livetec Systems Ltd, Building 52, Wrest Park, Silsoe, Bedford, MK45 4HS, UK

Short title: Novel percussive and cervical dislocation tools for despatching poultry

*Corresponding author: Dr Jessica Martin, The Royal (Dick) School of Veterinary Studies and The Roslin Institute, Easter Bush Campus, Edinburgh, Midlothian, EH25 9RG. Tel: +441316506062. Email: Jessica.Martin@ed.ac.uk

Abstract

1. Four mechanical poultry killing devices; modified Armadillo® (MARM), modified Rabbit Zinger™ (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.
2. 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.
3. 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.
4. The MPLI was inconsistent, and only performed optimally for 27.5% of birds, despite good killing potential when performing well. Severe crushing injury was seen in >50% of MPLI birds, suggesting that birds would die of asphyxia rather than cerebral ischemia, a major welfare concern. As a result the modified pliers are not recommended as a humane on-farm killing device for chickens.
5. This experiment provides important data on the killing potential of untried novel percussive and mechanical cervical dislocation methods, informing future studies.

Keywords

Killing; poultry; cervical dislocation; percussive; post-mortem; animal welfare.

Introduction

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 welfare on a large scale. Poultry may need to be killed on-farm for multiple reasons (e.g. injury, sickness and for stock management). Emergency killing on a large scale is often controlled by whole-house or containerised gas methods (e.g. Lambooij *et al.*, 1999; Gerritzen *et al.,* 2004; Gerritzen *et al.,* 2009; McKeegan *et al.,* 2011), but for the killing of smaller numbers of birds on-farm, there are currently two main methods: (i) cervical dislocation, which is designed to cause death by cerebral ischaemia and extensive damage to the spinal cord and brainstem (Ommaya & Gennarelli 1974; Gregory & Wotton 1990; Erasmus *et al*., 2010a,b; Bader *et al*., 2014; Martin *et al*., 2016); and (ii) percussive devices designed to cause extensive brain damage, resulting in brain death (Gregory & Wotton, 1990; HSA, 2004; Mason *et al.,* 2009; Erasmus *et al*., 2010a,b; Sparrey *et al*., 2014; Cors *et al.,* 2015).

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 to learn and perform, and does not require equipment (Mason *et al*., 2009; Sparrey *et al.,* 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 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 *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 brainstem through the action of stretching and twisting (Harrop *et al.,* 2001; Shi & Pryor, 2002; Pryor & Shi, 2006; Shi & Whitebone, 2006; Cartner *et al*., 2007; Erasmus *et al*., 2010a). 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 in poultry (Gregory& Wotton, 1986, 1990; Erasmus *et al*., 2010a), as well as other species (Tidswell *et al*., 1987; Cartner *et al*., 2007). Some studies have indicated that animals, 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 been noted that there is high variability in its application by different relevant groups (e.g. poultry stock-workers, veterinarians, trained slaughtermen) (Mason *et al.,* 2009; Sparrey *et al.,* 2014). Since January 2013 the use of manual cervical dislocation (MCD) as a killing method for poultry on-farm has been heavily restricted through the new EU legislation, 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 further research to explore current and novel methods for killing poultry in small numbers. Several mechanical devices have been developed recently (e.g. CASH Poultry Killer, Turkey Euthanasia Device) (Erasmus *et al*., 2010a; Erasmus *et al*., 2010b; HSA, 2004; Raj and O'Callaghan, 2001), however, none have been enthusiastically adopted across the commercial industry or by small poultry keepers.

Previous research has shown that post-mortem analysis is effective in inferring killing potential and time to loss of consciousness and has been used across several species in determining success rates of slaughter and on-farm killing method in livestock species while avoiding ethical concerns associated with the application of new killing methods (e.g. Anil *et al.*, 2002; 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 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 fractures, brain contusions, cerebral oedema, hemorrhaging and *contra-coup* damage (i.e. damage to the brain on both sides: the side that received the initial impact (coup) and the side 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 the application of killing methods. Determining the success rate of a killing device is essential 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 on-farm. 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.

Methods

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.

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 group to provide recommended environmental controls (Aviagen, 2009; Hy-Line, 2012). All birds were kept in floor pens with wood-shavings litter at significantly lower than commercial 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 and auditory contact with other birds within the same room. Broiler chicks and layer pullets were housed in group pens (L 1.5 m x W 2.5 m x H 1.5 m). Broilers (slaughter-age) and layer 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 maximum temperatures were recorded each morning.

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.

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.

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).

Four mechanical poultry killing devices: modified Armadillo® (MARM), modified Rabbit ZingerTM (MZIN), modified pliers (MPLI) and a novel mechanical cervical dislocation gloved device (NMCD) were assessed for their killing potential in cadaver birds (four bird type and age combinations). All methods developed are discussed in detail in Martin (2015) and were designed to comply with the current European legislation, EC1099/2009 (European Council, 2009). Briefly, the Armadillo® (Figure 1a) is a brain-stem penetrating device designed by a veterinarian to dispatch game birds in the field (Sparrey *et al.,* 2014; Martin, 2015). The device consists of a scissor-type mechanism (approximately 17 cm in length); the bird's head is placed into the 'cup' of the lower arm (beak facing downwards) and when ready to apply the operator squeezes the handles together, which pushes the top arm (and the penetrating spike) downwards into the back of the bird's skull, preferably through the foramen magnum therefore severing the top of the spinal cord (or brain stem), and causing death by cerebral ischemia. Presently there is no published scientific evidence on the efficacy of this device. Modifications (with the permission of the inventor) consisted of replacing the lower arm of the device in order 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 encompass chicken heads. Additional insertion cups were molded from 1mm thick plastic 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 and L=18 mm (layer pullets, broiler chicks)). The additional cups also had soft padding (Waxman 4719095N ½ 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 creating an oval shape for the upper opening.

The Rabbit Zinger™ (Pizzurro, 2009a,b) is a penetrating captive-bolt device originally designed to kill rabbits (Figure 1b). It uses the stored energy in rubber tubes to drive a 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 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™ (Pizzurro, 2009a) were used, which require 177 N to pull the bolt into the cocked position (Sparrey *et al.*, 2014; Martin *et al.,* 2016) and when fired the bolt (0.6 mm diameter) delivered approximately 11.87 J of kinetic energy. The modifications have been described previously (Martin, 2015; Martin *et al.,* 2016), but consisted of three aluminium appendages added to the base of the device in order to provide a method of gently restraining the bird's head: two rested either side 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 top-heaviness of the device when cocked.

'Semark' pliers (also known as the 'Humane Bird Dispatcher') weigh approximately 200 g and 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 is a slight gap between the blades (<1 mm). The pliers were modified (MPLI) in an attempt to 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). The edges of the blades remained blunt in order to reduce the risk of skin tearing and thus 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 dislocation, as the narrower blade would more easily slip between two cervical vertebra when force was applied. The blades were widened gradually to increase the size of the blade (over 3 mm) and therefore generate a dislocation (i.e. gap between the two vertebra), by pushing the vertebrae apart.

The NMCD device (Figure 1d) was designed to create a mechanical method for cervical 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 Multipurpose Stable Glove™, UK) designed to support the wrist and hand (and hypothesised to reduce strain injury in the operator) and a moveable metal insert. The metal insert consisted of two metal finger supports that were designed to fit around the bird's head to create a secure 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 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 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.

After device application, cadavers were immediately examined post-mortem in order to establish as accurately as possible the anatomical damage caused by the device. Specific 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, external blood loss and subcutaneous hematoma and the total number of attempts were recorded (e.g. multiple pulls for NMCD or miss-fire of MZIN). For the MZIN and MARM, seven specific measures were recorded: binary measures of damage to the skull, specific brain regions (left forebrain, right forebrain, cerebellum, midbrain and brainstem); and the presence of an internal brain cavity hematoma. For killing devices which caused trauma to the neck of 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 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. between C0-C1, C1-C2, C2-C3, etc.).The number of carotid arteries severed was also recorded as zero, one or both.

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.

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.

Statistical Analysis

All data were summarised in Microsoft Excel (2010) spread sheets and analysed using Genstat (14^{th} Edition). Statistical significance was based on F statistics and $P<0.05$ significance level. Summary graphs and statistics were produced at bird and treatment level. Generalised Linear Mixed Models (GLMM) (binomial distribution) were used to compare performance across the four killing devices in terms of kill potential and device success, while 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 performance were achieved by sub setting the data twice: initially to remove unsuccessfully "killed" birds (i.e. kill potential "no") in order to prevent data skewing; and then into two groups 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 the neck or the head. Statistical comparisons on anatomical measures were conducted via GLMMs (Poisson distribution and binomial distribution) or Linear Mixed Models (LLM) (normal distribution) dependent on the data distributions for each variable. Data transformations were attempted when necessary via Logarithm function. All models included batch number as random effects. All fixed effects were treated as factors and classed as categorical classifications and all interactions between factors were included in maximal models.

Results

A total of 36 birds were not successfully "killed" on the first attempt (NMCD = 0/40 birds; MPLI = 15/40 birds; MARM = 15/40 birds; and MZIN = 6/40 birds). Device had an effect on kill potential ($F_{(3,144)}$=2.88, *P*=0.038), with NMCD having the highest kill potential, with 100% of birds sustaining the required physical trauma to have caused death (Figure 3). The MARM 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 sustain the required physiological trauma to have resulted in death (mean = 0.87 ± 0.04), compared to older birds (mean = 0.68 ± 0.05). All other factors (bird weight, type and head measures) and their interactions had no effect on kill potential.

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.

Percussive methods

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 killing method is shown in Table 3. Killing device had no effect on the majority of post-mortem measures, apart from damage to left forebrain, mid brain, and brain stem. The MZIN was 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 sustaining damage compared to the MARM. No other factor or interaction affected external 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.

Cervical dislocation methods

For successfully killed birds (MPLI = 25/40 birds; NMCD = 40/40 birds), the percentage of birds for which the relevant neck trauma post mortem factor was present, according to killing method, is shown in Table 4. Numerically, MPLI was more likely to tear the skin, cause external bleeding, vertebral damage, trachea damage, and oesophagus damage compared to NMCD, but the differences were not significant. NMCD was more likely to cause cervical dislocation, 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 method ($F_{3,74}$=2.34, P=0.076), although there was a tendency ($P < 0.10$), for NMCD to be more likely to cause a higher level dislocation compared to MPLI (Figure 5).

Whether or not cervical dislocation (no = 0; yes = 1) occurred was significantly affected by bird type ($F_{1,74}$=5.98, P=0.014) and bird age ($F_{1,74}$=6.39, P=0.011), with dislocations more likely to occur in broilers (mean = 0.95 ± 0.05) rather than layers (mean = 0.55 ± 0.11), and younger birds (mean = 0.90 ± 0.07) compared to older birds (mean = 0.60 ± 0.11). The diameter of the birds' necks (N1) ($F_{1,74}$=4.00, P=0.050) also had an effect with unsuccessful dislocations associated with larger neck diameters (17.1±1.09 mm) compared to successful dislocations (14.9±0.51 mm). Bird type had an effect on the likelihood of vertebral damage (no = 0; yes = 1), with layers (mean = 0.75 ± 0.10) more likely to sustain damage than broilers (mean = 0.35 ± 0.11). No other factors or interactions, apart from killing method (reported above) had an effect on vertebral damage.

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).

Discussion

The results of this experiment provide important data to allow evaluation of the killing potential of four untried novel percussive and mechanical cervical dislocation methods for chickens. 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 shown to have the highest killing potential (100%), however, all devices achieved a killing 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 bird type. Device success was always lower than the killing potential for each method because 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 not always performing optimally, which could have welfare implications. For NMCD, the primary reason for this difference was the number of carotid arteries severed, as on occasion 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 damaged or only minor damage to all regions (e.g. internal brain cavity bleeding and bruising). Failures in device success with the MARM were primarily due to the spike not penetrating to 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 the cerebellum. In terms of brain trauma, this could reduce the chance of neurogenic shock and elongate the time to loss of consciousness and brain death (Alexander, 1995; Dumont *et al*., 2001; Freeman and Wright, 1953; White and Krause, 1993), but it did not appear to affect 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).

Post-mortem measures for the neck trauma methods highlighted that the MPLI caused numerically more instances (though not significant) of cause skin tears and external bleeding, 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; Halvorson and Hueston, 2006; Kingsten *et al*., 2005). The MPLI, designed to dislocate the 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 by the blades, would still be fatal, but would not necessarily cause death by cerebral ischemia, which is the intended outcome (Veras *et al*., 2000; Harrop *et al.,* 2001; Bader *et al.,* 2014). 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.

Both the MARM and MZIN always caused penetration of the skin and damage to the skull and 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 differently. With the MZIN, more than 60% of all birds received damage to the main areas of the brain (excluding the brain stem), demonstrating diffuse damage which the device is designed to cause in order to cause concussion and brain death (Alexander, 1995; Finnie *et al.,* 2000; Oppenheimer, 1968). The MZIN showed higher killing potential than the unmodified Rabbit Zinger^{TM}, which had previously been reported to have a kill success rate of 50% in poultry (DEFRA, 2014). The MARM caused focalised damage to the brain stem and cerebellum, highlighting that the modifications to the MARM had adequately adapted its design to more adequately fit poultry. Such damage to the brain stem theoretically would result in fatal functional impairment (e.g. puntilla method as described in Limon *et al.,* 2009; Limon *et al.,* 2010) (HSA, 2004; Morzel *et al*., 2002; Widjicks, 1995). The un-modified Armadillo® was tested previously (DEFRA, 2014), and was found to have a low kill success of 46%, therefore the higher kill potential could be attributed to the modifications or that the killing potential was tested on cadavers, which are easier to handle, improving application of the method. The increase in success in the MZIN could be attributed to the same reasons.

Other bird factors were shown to impact some post-mortem measures (e.g. dislocation level, vertebral damage), kill potential and device success, demonstrating inconsistency dependent on the target species, although their impact was more pronounced with the cervical dislocation methods than the head trauma methods. Bird age affected both killing potential and device 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 mature, and therefore bones and cartilage are less calcified and re-inforced, as well as 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 tissue, aging can have a detrimental effect, with reduced elasticity in arterial walls and skeletal muscle, reducing stretching potential, therefore carotid arteries and neck muscle are more 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 larger birds, therefore despite the increased elasticity, the magnitude of the stretch required to dislocate and tear should counteract this effect. In general, cervical dislocation was easier in broilers and younger birds, although these factors are confounded, as by definition broilers 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 larger necks (older birds). When considering vertebral damage, layers were more likely to receive damage, but again bird type was confounded with age, with laying hens being older than any other bird group. The increased likelihood of vertebral damage could also be attributed to brittle bones in the laying hens (Whitehead and Fleming, 2000). All other external factors had no impact on the post-mortem measures associated with brain trauma methods, indicating that these methods are less susceptible to inconsistency as when applied to various types, size and age of birds. However, this has to be taken within the context that both of the brain trauma methods: MZIN and MARM had killing potentials of 84.2% and 62.5% respectively, both of which highlight issues with reliability.

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 methods: NMCD, MARM and MZIN demonstrated killing potential, as well as consistency in 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-mortem evaluations would be desirable, for example, skull damage location and size of dislocation (i.e. measurement of gap between two dislocated vertebrae), in order to further 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 matching killing potential with the MARM. The abundant evidence of crushing injury in >50% of birds was also a major concern, especially as the new European legislation on the 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 not recommended as a humane on-farm killing device for chickens. The performance of the remaining three devices (NMCD, MZIN, MPLI) will be further assessed in live birds in order to establish their potential to provide a new humane method for despatching poultry on-farm.

Acknowledgements

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

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.

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.

Cartner, S.C., Barlow, S.C., Ness, T.J., (2007). Loss of Cortical Function in Mice After Decapitation, Cervical Dislocation, Potassium Chloride Injection, and CO2 Inhalation. *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.

DEFRA, (2015). DEFRA United Kingdom Poultry and Poultry Meat Statistics - March 2015, https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/423075/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.

Dumont, R.J., Okonkwo, D.O., Verma, S., Hurlbert, R.J., Boulos, P.T., Ellegala, D.B., Dumont, A.S., (2001). Acute Spinal Cord Injury, Part I: Pathophysiologic Mechanisms. *Clinical 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**: 1345-1354.

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 2009 on the Protection of Animals at the Time of Killing. In. ec.europa.eu/food/animal/welfare/slaughter/regulation_1099_2009_en.pdf. Accessed 28/09/2011.

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.

Finnie, J.W., Manavis, J., Blumbergs, P.C., Summersides, G.E., (2002). Brain damage in sheep from penetrating captive bolt stunning. *Australian Veterinary Journal*. **80**: 67-69.

Freeman, L.W., and Wright, T.W., (1953). Experimental Observations of Concussion and 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.

Gerritzen, M.A., Raj, A.B.M., (2009). Animal welfare and killing for disease control. In: 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

Grandin, T., (2010). Auditing animal welfare at slaughter plants. *Meat Science*. **86**: 56-65.

Gregory, N.G., Lee, C.J., Widdicombe, J.P., (2007). Depth of concussion in cattle shot by penetrating captive bolt. *Meat Science.* **77**: 499-503.

Gregory, N.G., Shaw, F., (2000). Penetrating Captive Bolt Stunning and Exsanguination of Cattle in Abattoirs. *Applied Animal Behaviour Science*. **3**: 215-230.

Gregory, N.G., Wotton, S.B., (1986). Effect of slaughter on the spantaneous and evoked 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**: 570-572.

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. *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.

Martin, J. E., McKeegan, D. E. F., Sparrey, J., and Sandilands, V., (2016). Comparison of 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.

Nair, K.S., (2005). Aging muscle*. The American Journal of Clinical Nutrition* **81**: 953-963.

Ommaya, A.K., Gennarelli, T.A., (1974). Cerebral concussion and traumatic unconsciousness. *Brain* **97**: 633-654.

Oppenheimer, D.R., (1968). Microscopic lesions in the brain followings head injury. *Journal Neurology, Neurosurgery & Psychiatry* **31**: 299-306.

Parent, A., Harkey, L.H., Touchstone, D.A., Smit, E.E., Smith, R.R., (1992). Lateral Cervical Spine Dislocation and Vertebral Artery Injury. *Neurosurgery* **31**: 501-509.

Pizzurro, S., (2009a). About us - expectation of order fulfilment. United States.

Pizzurro, S., (2009b). Zinger Stun Guns™ - The Rabbit Zinger™, (TRZ001). United States.

Pryor, J.D., Shi, R., (2006). Electrophysiological changes in isolated spinal cord white matter in response to oxygen deprivation. *Spinal Cord* **4**: 653-661.

Raj, A.B.M., O'Callaghan, M., (2001). Evaluation of a pneumatically operated captive bolt for stunning/killing broiler chickens. *British Poultry Science* **42**: 295-299.

Salim, A., Martin, M., Sangthong, B., Brown, C., Rhee, P., Demetriades, D., (2006). Near-hanging 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.

Veras, L., Pedraza-Gutiérrez, S., Castellanos, J., Capellades, J., Casamitjana, J., Rovira-Cañellas, A., (2000). Vertebral Artery Occlusiion After Acute Cervical Spine Trauma. *Spine* **25**: 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.

Whitehead, C.C., Fleming, R.H., (2000). Osteoporosis in Cage Layers. *Poultry Science* **79**: 1033-1041.

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	**N**	**Mean bird age at killing (days)**	**Mean bird weight at killing (kg)**	**Housed stocking density (kg/m^2)**
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 ± 0.1	0.7 ± 0.2	1.9
Broiler (slaughter age)	40	37.1 ± 0.6	1.9 ± 0.7	5.1

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 • Severe damage to a minimum of one area of the brain
MPLI	• Complete cervical dislocation at C0-C1 • Severing of the top of the spinal cord (i.e. brain stem) • Severing of both carotid arteries • No breakage to the skin • No crushing injury to the trachea or oesophagus
NMCD	• Complete cervical dislocation at C0-C1 • Severing of the top of the spinal cord (i.e. brain stem) • Severing of both carotid arteries • No breakage to the skin

Table 3: Percentage of birds killed successfully for which the relevant head trauma post mortem factor was present, according to killing method. Significant P values are underlined.

	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	0.013
Brain stem damage	31.3	92.0	5.10	0.034

Table 4: Percentage of birds killed successfully for which the relevant neck trauma post mortem factor was present, according to killing method. Significant P values are underlined.

Post mortem measure	**Percentage of birds**		***F statistic***	***P value***
	NMCD	**MPLI**		
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	0.012
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

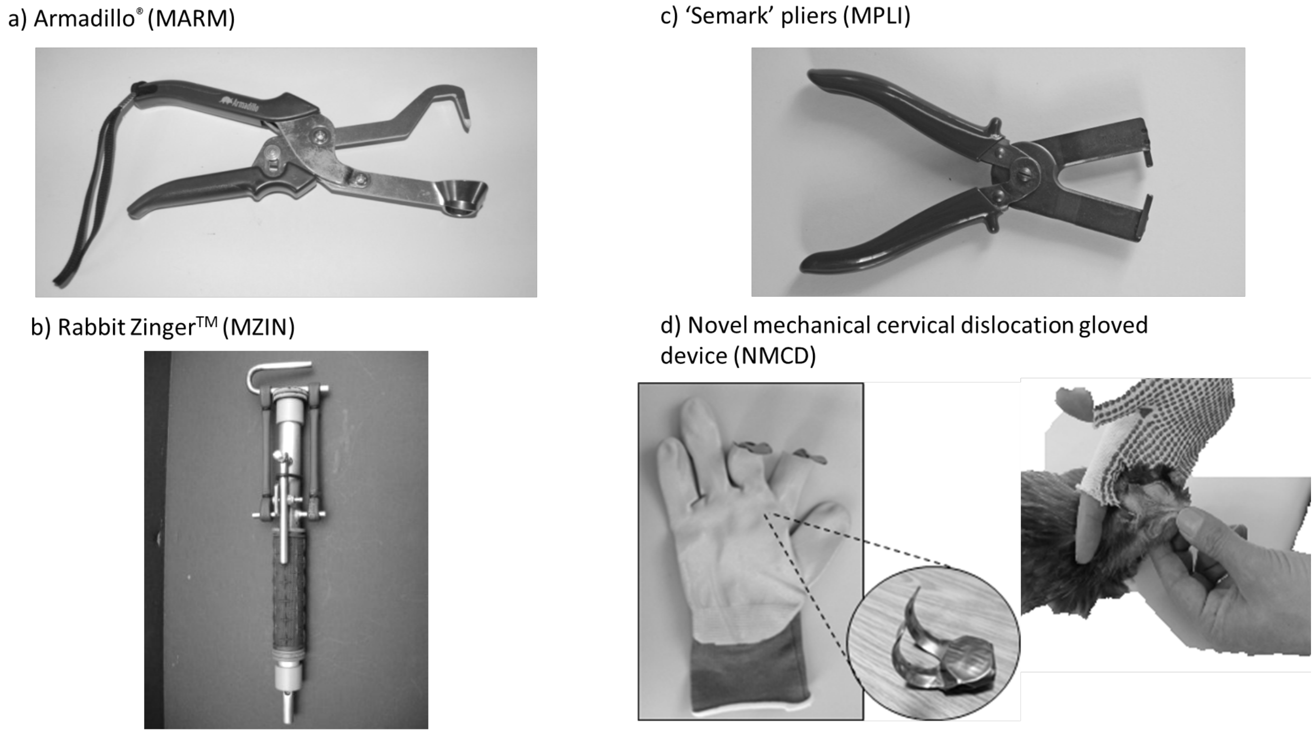

Figure 1: Photographs of tested devices: a) Armadillo®, b) Rabbit Zinger™, c) 'Semark' pliers, and d) the Novel mechanical cervical dislocation gloved device.

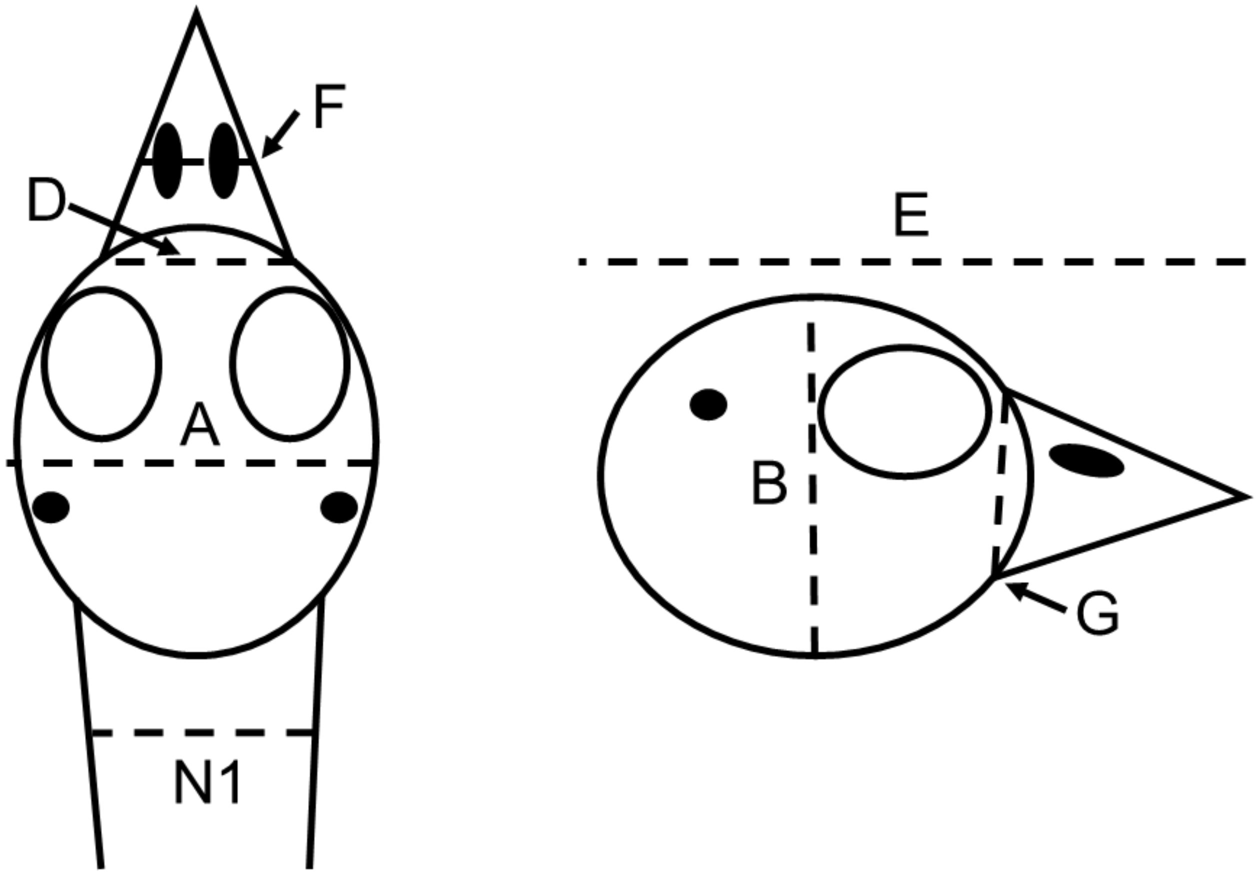

Figure 2: Schematic showing head and neck measures: A = width of head; B = lower mandible to top of skull; D = width of base of beak; E = base of skull to front of beak; F = width of beak at central nostril level; G = depth of beak; and N1 = width of neck.

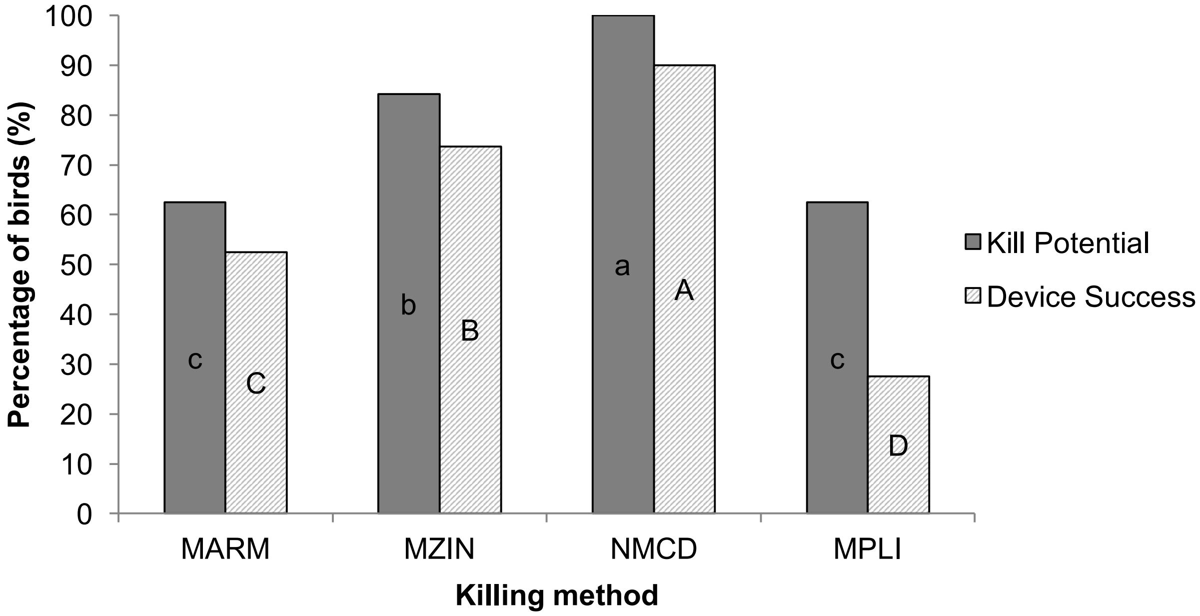

Figure 3: Summary of kill potential and device success rates (%) across the four killing devices. No common lettering indicates that there is a significant difference between the groups.

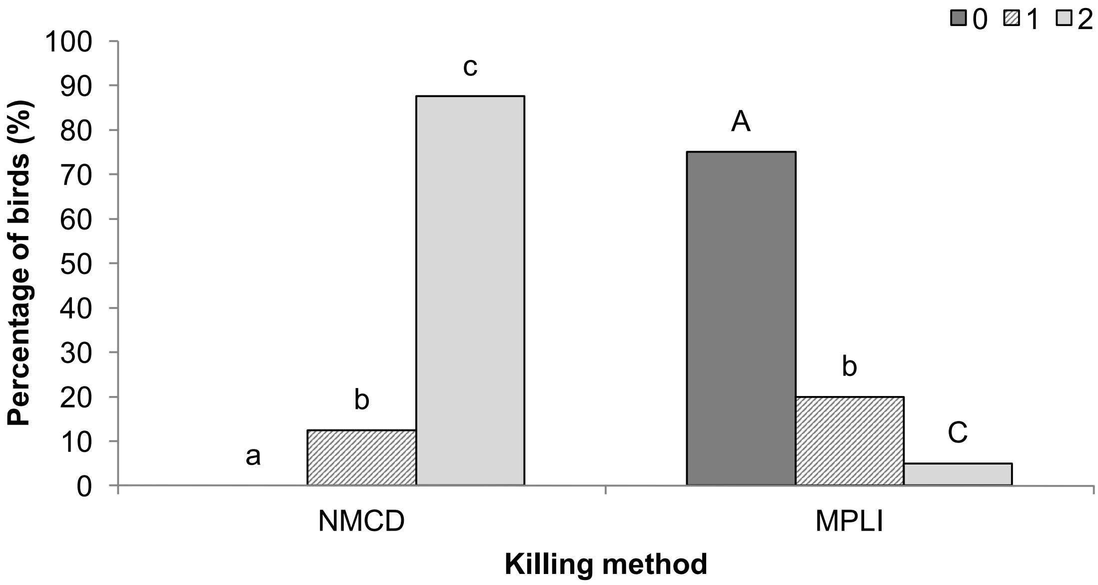

Figure 4: Percentage of birds by the number of carotid arteries severed dependent on killing method. No common lettering indicates that there is a significant difference between the groups.

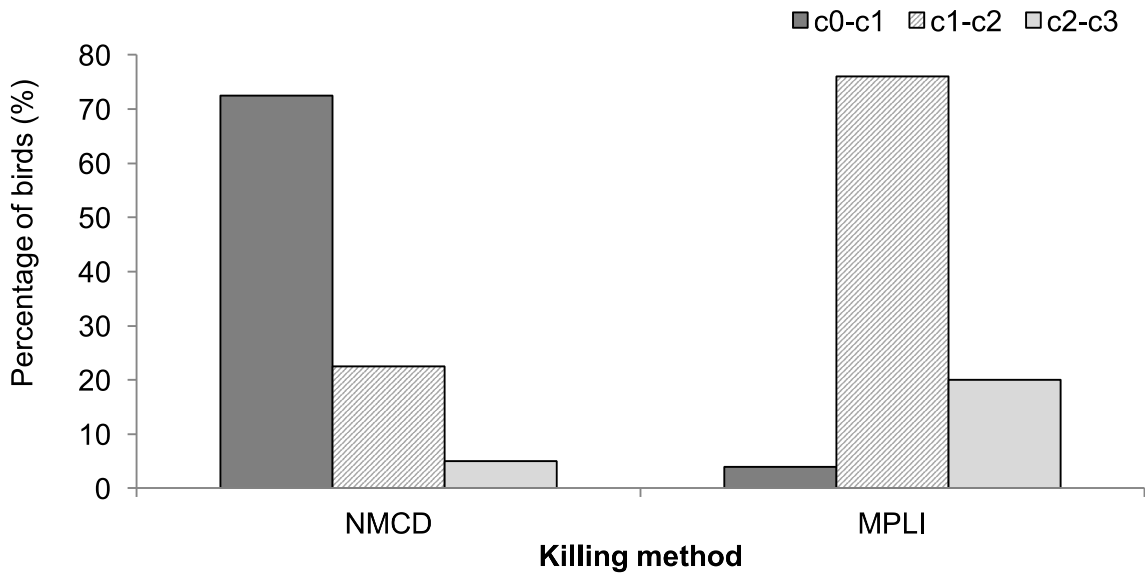

Figure 5: Distribution of birds by the various dislocation levels in relation to killing method.