Efficacy of three different cervical dislocation methods for on-farm killing of layer chicks

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ABSTRACT Unfit chicks with low viability are often euthanized in the layer industry. An effective euthanasia protocol is characterized by rapid, irreversible insensibility, followed by prompt death. This study was conducted to evaluate the efficacy of three cervical dislocation methods for killing layer chicks (2−3-day-old, avg BW ± SD; 44 ± 3 g, n = 40): manual cervical dislocation (**CD**), assisted manual cervical dislocation (**ACD**; the bird's ventral neck is placed on a blunt table edge and the back of the neck pressed firmly), and mechanical cervical dislocation by Koechner Euthanizing Device (**KED**-model-S). All three killing methods were assessed on anesthetized chicks (intramuscular injections of medetomidine [0.3 mg/kg BW] and ketamine [30 mg/kg BW] were used to induce clinical anesthesia). CD and ACD were also evaluated using conscious chicks to compare the killing methods and to determine the effect of anesthesia on response variables. There were no differences in time to loss of pupillary light reflex, cessation of heartbeat, or duration of gasping between conscious chicks killed with CD and ACD, but these values were all longer for conscious compared to anesthetized chicks. KED resulted in longer latencies to loss of pupillary light reflex, cessation of heartbeat, and duration of gasping.
Radiographs revealed that both CD and ACD resulted in cervical luxation, mainly below the C4 vertebra, whereas KED did not cause luxation in any of the 8 chicks tested. Chicks killed by CD and ACD presented more subdural hemorrhage (**SDH**) at the site of cervical dislocation than those killed by KED. None of the killing methods resulted in brain trauma. Compared to CD and ACD, KED resulted in longer latency to brain death and less anatomical pathology indicating a lower efficacy of KED as an on-farm killing method.

Key words: anesthesia, brain death, cervical dislocation, chicks, euthanasia

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INTRODUCTION

While it is often necessary to euthanize young chicks due to low viability, little research has investigated the efficacy of different physical on-farm euthanasia methodologies. Manual cervical dislocation (**CD**) is often used to kill poultry on-farm due to its practicality, although application of this method in young chicks can be difficult due to their smaller necks and heads, which can result in decapitation, and may be esthetically unacceptable to the operators. Small birds including chicks and poults can also be killed by an assisted technique for manual cervical dislocation whereby their neck is pressed against a bar or edge of a table (PIC, 2016). Alternatively, there are mechanical cervical dislocation methods, such as using the blunt edge of pair of scissors or pliers, but the efficacy of these methods has only been assessed in laboratory mice (Carbone et al., 2012). Mechanical cervical dislocation devices are manufactured and commercially available for poultry, but their ability to kill birds is equivocal, as Woolcott et al. (2018) found that the Koechner Euthanizing Device (**KED-S**) was ineffective at dislocating the vertebrae and severing the spinal cord of turkey poults. The AVMA (2020) states that "cervical dislocation must result in luxation of the cervical vertebrae without primary crushing of the vertebrae or spinal cord".

Thus, the aim of this study was to scientifically assess the efficacy of 3 methods of cervical dislocation for on-

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farm killing of layer chicks: manual cervical dislocation, assisted manual cervical dislocation (pressing neck against a blunt surface) and mechanical cervical dislocation (using KED-S). Methods were compared using brain stem reflexes, behavioral responses, and postmortem measures of brain and vertebral damage. Due to ethical concerns, novel killing devices are often evaluated initially on cadavers or with anesthetized animals (Martin et al., 2017; Woolcott et al., 2018; Bandara et al., 2019a,b) as anesthetic reduces potential distress and pain associated with killing method (Alkire et al., 2008). Thus, this study assessed the KED-model-S in anesthetized chicks. However, whereas some brain stem reflexes and behaviors commonly used to assess euthanasia methods are present under anesthesia in poultry, others are not (Sandercock et al., 2014; Woolcott et al., 2018; Bandara et al., 2019a). Therefore, a secondary objective of this study was to determine the effect of the anesthetic agent on antemortem measures commonly used to assess killing methods in birds, including brain stem reflexes and behavioral responses.

MATERIALS AND METHODS

The protocol and procedures for this research were reviewed and approved by the University of Guelph Animal Care Committee (**ACC**) under Animal Utilization Protocol # 3321. The University of Guelph holds a Good Animal Practice certificate issued by the Canadian Council on Animal Care.

Animals and Facilities

A total of 40 healthy chicks (Avg BW ± SD; 44 ± 3 g, age 2−3 days old) from three strains (ISA Brown, Shaver White, Lohmann Select Leghorn-lite) were sourced from the Arkell Poultry Research Station at the University of Guelph and randomly assigned to one of 5 treatment groups; manual cervical dislocation (CD), anesthetized manual cervical dislocation (**aCD**), assisted manual cervical dislocation (**ACD**), anesthetized assisted manual cervical dislocation (**aACD**) or anesthetized mechanical cervical dislocation by KED (**aMCD**). The three strains were balanced across the treatments. Table 1 shows strain, sex, body weight, and sample sizes for the different treatment groups used in the study. The chicks were obtained from different research projects, were surplus and targeted for euthanasia. The experiment was conducted over 2 consecutive trial days and the order of application of assigned treatments on a trial day was determined using the random number generator in Excel.

Anesthesia and Killing Procedures

Anesthesia was induced in the birds by using a combination of medetomidine (1 mg/ml) (CepetorTM, DIN:02337177, Modern Veterinary Therapeutics, LLC, Miami, FL) and ketamine (100 mg/mL) (Ketaset, DIN: 02173239, Pfizer Animal Health, Kirkland, QC, Canada). A pilot study was conducted to determine the appropriate drug doses to induce surgical anesthesia in chicks of a similar age to the experimental ones. The chosen doses (medetomidine = 0.3 mg/kg body weight; ketamine = 30 mg/kg body weight) were administered as separate injections into the breast muscle by a veterinarian monitoring the anesthesia.

Following anesthetic administration, the chicks were kept in a crate in a quiet and dark room until the anesthetic took effect. Fifteen minutes after drug application, chicks were assessed for breathing, pupillary light reflex, pedal reflex, neck muscle tone, jaw tone, and heartbeat. The same responses were checked directly prior to application of the killing method. Birds were determined to be ready to apply the killing method when they were nonresponsive to handling, and when assessment of breathing pattern, heart auscultation, jaw tone, and pedal reflex indicated surgical anesthesia.

Manual cervical dislocation (CD) was performed by a trained and experienced technician. The chick's head

Table 1. Strain, sex, body weight, and sample sizes for the different treatments used in the study.

Killing method	Total number of chicks	Body weight (g) (Avg wt ± SD)[1]	Strain	Sex: Male	Sex: Female
CD	8	44 ± 5	ISA Brown,	2	0
			Shaver White,	1	2
			Lohmann Select Leghorn-lite	1	2
aCD	8	45 ± 3	ISA Brown,	1	1
			Shaver White,	1	2
			Lohmann Select Leghorn-lite	1	2
ACD	8	44 ± 2	ISA Brown,	1	1
			Shaver White,	1	2
			Lohmann Select Leghorn-lite	1	2
aACD	8	44 ± 3	ISA Brown,	1	1
			Shaver White,	0	3
			Lohmann Select Leghorn-lite	1	2
aMCD	8	44 ± 2	ISA Brown,	1	1
			Shaver White,	1	2
			Lohmann Select Leghorn-lite	1	2

Abbreviations: CD, conscious manual cervical dislocation; aCD, anesthetized manual cervical dislocation; ACD, conscious assisted manual cervical dislocation; aACD, anesthetized assisted manual cervical dislocation; aMCD, anesthetized mechanical cervical dislocation by KED.

[1]Average weight ± Standard deviation.

was held in the operator's palm, with the neck between the index finger and thumb. Manual cervical dislocation was performed in one swift movement with the operator pulling down on the chick's head, stretching the neck, while rotating the chick's head upward into the back of the neck (Figure 1A). Both manual and assisted manual cervical dislocation (ACD) were performed by the same trained technician. The neck of the chick was pressed against the blunt end of a desk (90 degree angle) by the technician's thumbs, while the chick's body was supported by the palm and fingers (Figure 1B). The KED is manufactured as a mechanical cervical dislocation device for poultry by Koechner MFG. CO., INC (2016) (U.S. Patent No. 8,152,605). The KED was applied according to the manufacturer's instructions (Koechner MFG. Co., INC. 2016). All of the birds were manually restrained on a table in a sternal recumbent position when applying the KED. The double angle blade was placed under the neck of the bird while the single side blade was placed dorsally above the top of the neck at the base of the skull. The handles were brought together quickly and firmly to cause dislocation (Figure 1C). Then the device was removed from the neck of the bird. The model-S which was used in the current study is designed for birds weighing up to 1.8 kg (Figure 2).

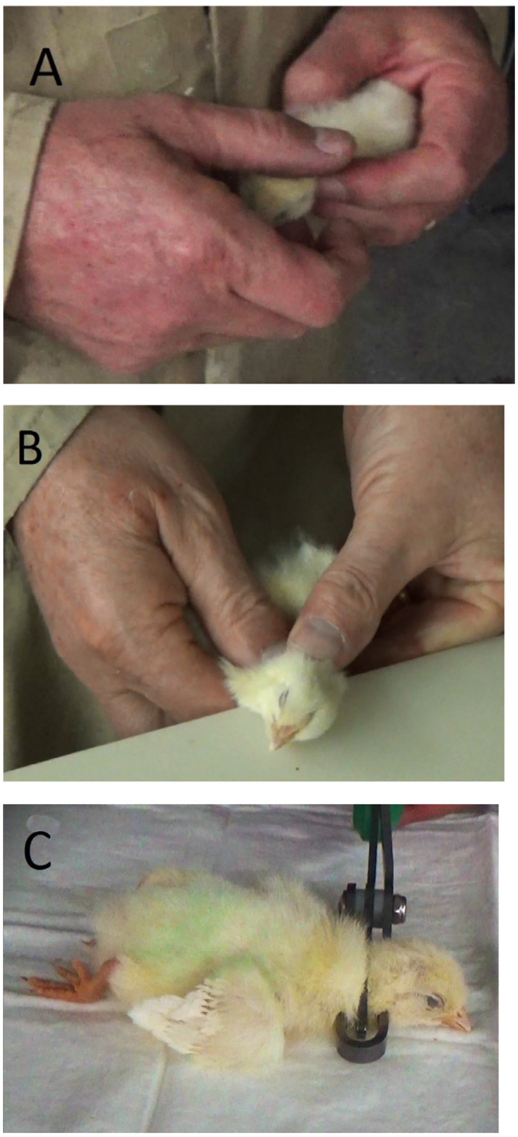

Figure 1. Three different killing methods of layer chicks. (A) Manual cervical dislocation. (B) Assisted manual cervical dislocation. (C) Mechanical cervical dislocation by KED-model-S.

Figure 2. The mechanical cervical dislocation device used in the experiment, Koechner Euthanizing Device (KED), model-S.

Antemortem Assessment

Immediately after application of a killing method and then every 10 s until cessation, chicks were observed for presence or absence of pupillary light reflex, gasping (paroxysmal opening of the beak), clonic convulsions (rapid, uncoordinated movement of the body and wings), and cardiac arrest (by auscultation by using a stethoscope) (Bandara et al., 2019a,b). A single observer who was not blind to treatment collected all data and subsequently confirmed the time of each event from video recordings that commenced immediately after the application of a killing method. Unlike what is observed in adult layer chickens, nictitating membrane reflex, feather erection, tonic convulsion, and cloacal relaxation were absent or difficult to observe in the chicks. Thus, these measures were excluded from the study.

Assessment of Radiographs

Radiographs of each chick were performed immediately following euthanasia using the same methodology as Bandara et al. (2019a). A board-certified veterinary radiologist blinded to the treatment assessed the radiographs for site of luxation/subluxation and for presence, type, and site(s) of fractures (Table 5).

Macroscopic Assessment of Tissue Damage

Degree of external damage and bleeding caused by each killing method was assessed (0−2 scale) based on laceration of the skin and the presence of external hemorrhage on the neck: 0 = no laceration of the skin, 1 = laceration of the skin with no external bleeding, and

2 = laceration of the skin with external bleeding (Bandara et al., 2019a).

Degree of injury caused by each killing method was assessed based on degree of subcutaneous hemorrhage (**SCH**) at the site of cervical dislocation (0 = none to 4 = >75% of surface affected), damage to the trachea (yes/no), transection of the spinal cord (yes/no), and degree of subdural hemorrhage (**SDH**) on the brain (0 =none to 4 = >75% of the surface affected) using the same procedure described in Bandara et al. (2019b).

Microscopic Assessment of Brain Trauma

Brains were collected from six randomly selected chicks in each killing method and placed in 10% buffered formalin for at least 14 days before trimming. Three sections of the brain (cerebrum, mid brain and thalamus, and cerebellum/hind brain) were trimmed and sampled by one individual for consistency (Woolcott et al., 2018; Bandara et al., 2019a,b). The tissue sections were embedded in paraffin, cut at 4 μm, and stained with hematoxylin and eosin using standard techniques to make microscopic slides (Animal Health Laboratory, University of Guelph). The sections on the slides were assessed microscopically by a veterinary pathologist blinded to the treatments to determine the degree of SDH and parenchymal hemorrhage (**PCH**). The degree of SDH and PCH were scored for each microscopic slide using the same procedure as Woolcott et al. (2018) and Bandara et al. (2019a,b).

Statistical Analyses

Statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC). Three separate statistical analyses were performed using the same model described below. The first analysis was used to compare the behavioral responses and reflex variables of conscious chicks killed by CD and ACD. The second analysis was used to compare anesthetized chicks to conscious chicks (CD vs. aCD and ACD vs. aACD), to determine the effects of anesthesia on each outcome variable. In the third analysis, the response variables of the anesthetized chicks were statistically compared among the 3 killing methods [aCD, aACD, aMCD].

Generalized linear mixed models (**GLMM**) were used to analyze the fixed effect of the killing method on the selected antemortem measurements (time to loss of pupillary light reflex, duration of gasping, time at last movement, time to cessation of heartbeat). A least significant means separation was conducted by using the Tukey−Kramer test.

Generalized linear mixed models (GLMM) with multinomial distribution and cumulative logit link functions were used to analyze the effect of the killing method on postmortem macroscopic SCH at the site of dislocation, microscopic SDH at the dislocation site in the neck (multinomial ordinal data). Odds ratios were computed to compare differences in the levels of fixed effects. Dislocation site was analyzed categorically.

RESULTS

Conscious Chicks: CD vs. ACD

There were no differences ($P = 0.3918$) between conscious chicks killed by CD (91.6 ± 6) and conscious chicks killed by ACD (98.7 ± 6) in time to loss of pupillary light reflex, gasping ($P = 0.5894$, CD 85.6± 15, ACD 98.1 ± 15), or cessation of heartbeat ($P = 0.3071$, CD 162.5 ± 10, ACD 176.7 ± 10). However, there was a significant difference ($P = 0.0137$) in time at last movement between conscious chicks killed by CD (113.6 ± 10 s) and conscious chicks killed by ACD (143.3 ± 10 s).

Conscious vs. anesthetized chicks (CD vs. aCD and ACD vs. aACD)

Pupillary light reflex was observed in all conscious and anesthetized chicks before and after applying the killing method (CD or ACD). Convulsions were absent in 96% of anesthetized chicks prior to killing method. Thus, duration of convulsions or the time at last movement were not used to assess differences in killing methods in anesthetized chicks.

The times to cessation of pupillary light reflex, heartbeat, and the duration of gasping in conscious and anesthetized birds killed by CD or ACD are presented in Table 2. There was an effect of anesthesia on time to loss of pupillary light reflex in the chicks killed by CD ($P = 0.048$) and ACD ($P = 0.008$), with longer time in conscious chicks than in anesthetized chicks. A longer latency to cessation of heartbeat was observed after ACD compared to aACD ($P = 0.023$). Similarly, there was a tendency for longer latency to cessation of heartbeat in chicks killed by CD compared to aCD ($P = 0.0624$). Anesthesia also affected the duration of

Table 2. Mean latencies to or durations of (± SE s) antemortem measures in conscious and anesthetized chicks killed by manual cervical dislocation (CD vs. aCD) and assisted manual cervical dislocation (ACD vs. aACD).

Measure	Conscious	Anesthetized	*P* value
Manual cervical dislocation	CD (N = 8)	aCD (N = 8)	
Time to loss of pupillary light reflex (s)	91.6 ± 6.30	66.8 ± 7.30	0.0487
Gasping duration (s)	85.6 ± 12.21	28.2 ± 13.06	0.0020
Time to cessation of heartbeat (s)	162.5 ± 10.46	141.5 ± 11.26	0.0624
Assisted manual cervical dislocation	ACD (N = 8)	aACD (N = 8)	
Time to loss of pupillary light reflex (s)	98.8 ± 7.73	76.8 ± 7.73	0.0080
Gasping duration (s)	98.1 ± 16.46	72.4 ± 17.33	0.1941
Time to cessation of heartbeat (s)	176.8 ± 13.34	137.9 ± 13.34	0.0230

Abbreviations: CD, conscious manual cervical dislocation; aCD, anesthetized manual cervical dislocation; ACD, conscious assisted manual cervical dislocation; aACD, anesthetized assisted manual cervical.

P values are given for effects of anesthesia.

Table 3. Mean latencies to or durations of (± SE s) antemortem measures in anesthetized chicks killed by manual (aCD), assisted manual (aACD) and mechanical cervical dislocation (aMCD).

Measure	aCD (N = 8)	aACD (N = 8)	aMCD (N = 8)	*P* value
Time to loss of pupillary light reflex (s)	66.7 ± 9.03	76.7 ± 7.80	94.4 ± 7.81	0.0962
Gasping duration (s)	32.2 ± 16.09[b]	75.7 ± 14.90[a,b]	103.4 ±13.93[a]	0.0210
Time to cessation of heartbeat (s)	138.5 ± 18.05[b]	137.9 ± 15.63[b]	196.4 ±15.63[a]	0.0366

Abbreviations: aCD, anesthetized manual cervical dislocation; aACD, Anesthetized assisted manual cervical dislocation; aMCD, anesthetized mechanical cervical dislocation by KED.

[ab]Different superscript letters indicate ($P < 0.05$) differences between means. *P* values are given for effects of method.

gasping in the chicks killed by CD, with a longer duration in CD compared to aCD ($P = 0.002$). However, the duration of gasping did not differ between chicks killed by ACD or aACD ($P = 0.1941$; Table 2).

Anesthetized Chicks (aCD, aACD, and aMCD)

Reflex and behavioural responses and their mean latencies of chicks killed by aCD, aACD and aMCD are shown in Table 3. There was a tendency for a longer latency to loss of pupillary light reflex in aMCD compared to aCD and aACD ($P = 0.0962$). All chicks except one (killed by aACD) gasped after application of killing method. The average duration of gasping was longer in aMCD than aCD ($P = 0.021$).The average duration of gasping in aCD did not differ from the chicks killed by aACD, while aACD did not differ from aMCD. A longer latency time to cessation of heartbeat was observed in the chicks killed by aMCD ($P = 0.036$) compared to aCD or aACD.

Results of radiographic scoring for the presence and site of cervical dislocation are presented in Table 4. Luxation or subluxation were absent in all chicks killed by aMCD**.** However, all chicks killed by CD, aCD, ACD, or aACD had luxation whether or not they were conscious or anesthetized when the method was applied. Subluxation was found in only one chick (from the CD treatment). The site of dislocation varied for different killing methods ($P = 0.007$). The ideal dislocation site of skull to C1 was absent for all the killing methods. Dislocation was found below the 4th vertebra in all chicks killed by either CD or ACD. Most chicks (68%, conscious and anesthetized) killed by manual CD were dislocated between the C5 and C6 vertebra. Similarly, all the chicks except one (conscious and anesthetized) killed with ACD had dislocation in between C4 and C5 or C5 and C6 vertebrae. Examples of radiographs demonstrating cervical dislocation sites in chicks are shown in Figure 3. Fractured vertebrae were found in 10% of the birds, with one bird in each of the CD, aCD, ACD, and aACD treatments. These fractures occurred at various locations between C2 and C7. aMCD did not cause any fractures.

Gross macroscopic examination indicated spinal cord transection in all chicks killed by CD and ACD irrespective of conscious or anesthetized state (Table 5). However, an intact spinal cord was observed in all chicks killed by aMCD. No gross damage to the trachea could be detected with any killing method. There was no effect of killing method on external damage to the neck ($P = 0.4305$). Subcutaneous hemorrhage (SCH) in the neck was different among the killing methods ($P = 0.007$; Table 5). Greater scores for the SCH hemorrhage were observed in the chicks killed by CD and ACD than in the chicks killed by aMCD. There was no difference in the degree of SCH between the chicks killed by CD and ACD. Except for one, all chicks killed by aMCD had minimum SCH. Macroscopic subdural hemorrhage in the brain was not observed in any chick.

Microscopic examination of brain sections indicated that none of the chicks killed by any method had any subdural hemorrhage or parenchymal hemorrhage in any of the 3 sections (cerebrum, mid brain and thalamus, and cerebellum/hind brain) of the brain.

Table 4. Presence and location of luxation (from radio graphs) and spinal cord transection (by macroscopic assessment) in conscious and anesthetized chicks killed by 3 different killing methods. Values indicate number of birds[1].

		Luxation						No luxation	Spinal cord transected
Treatment	N	C2-C3	C3-C4	C4-C5	C5-C6	C6-C7	C7-C8	No. of chicks	No. of chicks
CD	8	0	0	0	1	5*	2	1	8
aCD	8	0	0	0	1	6	1	0	8
ACD	8	1	0	4	3	0	0	0	8
aACD	8	0	0	3	5	0	0	0	8
aMCD	8	0	0	0	0	0	0	8*	0

[1]Statistical analysis is given in the text. Results of conscious chicks and anesthetized chicks were pooled for manual cervical dislocation and assisted manual cervical dislocation in statistical analysis.

*One conscious chick killed by CD had both luxation (C6-C7) and sub luxation (C5-C6). CD, Conscious manual cervical dislocation; aCD, Anesthetized manual cervical dislocation; ACD, Assisted manual cervical dislocation; aACD, Anesthetized assisted manual cervical dislocation; aMCD, Anesthetized mechanical cervical dislocation by KED.

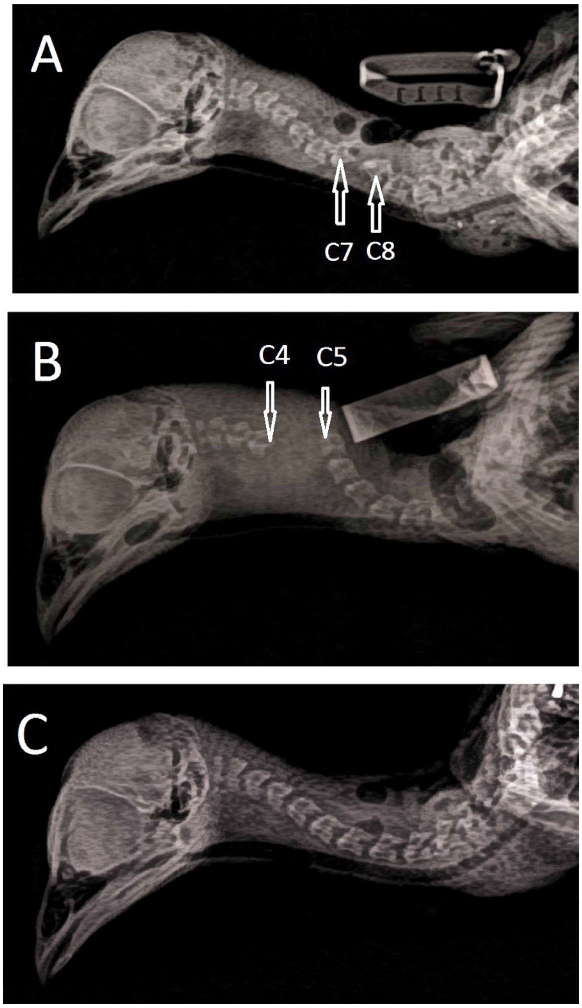

Figure 3. Radiographs of chicks showing cervical dislocations. (A) Dislocation between the 7th (C7) and 8th (C8) cervical vertebra in a chick killed by manual cervical dislocation; (B) Dislocation between C4 and C5 in a chick killed by assisted manual cervical dislocation; (C) Intact cervical vertebrae of a chick killed by mechanical cervical dislocation by KED.

DISCUSSION

To our knowledge, this is the first report on the efficacy of different physical methods of killing for young layer chicks. One of the main findings of this study was that the mechanical cervical dislocation device (specifically, KED-S) was ineffective at causing luxation of the vertebrae and transecting the spinal cord. However, none of the methods resulted in the ideal site for dislocation. Ideal cervical dislocation separates the cervical vertebrae in between the skull and the atlas (C1) while completely transecting the spinal cord and disrupting blood vessels (Martin et al., 2017; AVMA, 2020).

Table 5. Macroscopic evaluation of subcutaneous hemorrhage on the neck. Number of birds with each score are indicated.

Method	Score					*P* value
	0	1	2	3	4	
Manual cervical dislocation[1]	1	1	9	3	2	0.007
Assisted manual cervical dislocation[1]	0	3	4	5	4	
Mechanical cervical dislocation by KED	1	7	0	0	0	

[1]Data of conscious and anesthetized birds were pooled for manual cervical dislocation and assisted manual cervical dislocation in the statistical analysis.

Cranial dislocation was associated with rapid loss of reflexes followed by successful death in chickens compared to caudal dislocations (Martin et al., 2018). Carbone et al. (2012) reported that rapid respiratory arrest resulted in cervically dislocated mice with radiographically visible lesions in the high cervical or atlanto-occipital region. In the current study, both CD and ACD resulted in dislocation at a lower cervical vertebral region. This contrasts with studies in adult layer chickens where CD resulted in most dislocations between the skull and atlas (C1) (Bader et al., 2014; Martin et al., 2016; Bandara et al., 2019a).

One of the concerns with mechanical devices for cervical dislocation is fractures to the vertebrae (AVMA, 2020). A few chicks killed by CD or ACD demonstrated cervical bone fractures which was in agreement with our previous study using adult layer chickens (Bandara et al., 2019a). However, no birds tested with aMCD in the current study had fractures, in contrast to research with adult laying hens (using KED-C; Bandara et al., 2019a), 3-wk-old turkeys (using KED-S; Woolcott et al., 2018) and 8-day-old broiler chickens (using KED-S; Baker-Cook et al., 2021). The current results are similar to the results of Woolcott et al. (2018) in which none of the five 1-wk-old turkey poults tested with the KED-S demonstrated dislocation or spinal cord transection, and this study we noted that none of the turkey poults were successfully euthanized as a result of the KED application. In contrast, Baker-Cook et al. (2021) found that all 1-wk-old broiler chickens killed with KED-S had dislocations (at C2-C3 or lower) and death was as a result of the application. There was considerable variation in the size of the birds used in this and other studies with layer chicks in the current study averaging 44 g, and turkey poults (Woolcott et al., 2018) averaging between 40 and 60 g in body weight and broiler chicks (Baker-Cook et al., 2021) averaging 150 g. Therefore, the effectiveness of MCD devices cannot be generalized across different models of device or across sizes and species of birds.

The KED-S is a novel mechanical cervical dislocation device and the current study was the first to assess its efficacy in layer chicks. Due to ethical concerns, general anesthesia was induced using a combination of ketamine and medetomidine to alleviate any potential pain associated with the killing method. This drug combination is often used for avian anesthesia (Paul-Murphy and Fialkowski, 2001). Our research with adult laying hens showed this anesthetic protocol to mitigate some brain stem reflexes, behavioral responses, and physiological parameters (Bandara et al., 2019a). Therefore, we studied the effect of anesthesia by comparing conscious and anesthetized chicks using the 2 methods of manual CD. Pupillary light reflex was present, but it diminished sooner in the anesthetized chicks. This result was similar to a finding with adult laying hens using the same anesthetic protocol (Bandara et al., 2019a). However, Woolcott et al. (2018) reported no difference in cessation of pupillary light reflex in anesthetized vs. conscious turkeys using the same anesthetic protocol. The anesthetics used in the current study eliminated or reduced clonic and tonic convulsions. Similarly, previous studies also reported a reduction or elimination of some behavioural responses and reflexes with different anesthetics and sedatives (Sandercock et al., 2014; Woolcott et al., 2018; Bandara et al., 2019a), suggesting that convulsions, nictitating membrane reflex and cloacal relaxation are not appropriate measures of time to brain death in cases where ketamine and medetomidine have been administered in chickens.

We found no difference in time to loss of pupillary light reflex between CD and ACD, indicating no difference in time to brain death between the methods. There was also no difference in the duration of gasping and time to cessation of heartbeat between CD and ACD, but time at last movement was later with ACD. Time of last movement has been identified as a good indicator of clinical death in layer chickens for on-farm situations (Bandara et al., 2019b).

Latency to brain stem death was assessed based on latency to loss of pupillary light reflex. There was a trend for longer time to loss of pupillary light reflex with aMCD compared to aCD and aACD. Moreover, chicks killed with aMCD had a longer duration of gasping and longer latency to cessation of heartbeat compared to aCD and aACD, indicating a longer latency to clinical death in the chicks killed by aMCD. Other studies also revealed longer latencies to loss of eye reflexes in poultry killed by MCD using the KED compared to CD (broilers, Baker-Cook et al., 2021; laying hens, Bandara et al., 2019a; broilers, Jacobs et al., 2019; turkeys, Woolcott et al., 2018; laying hens, Hernandez et al., 2019; chickens, Gregory and Wotton, 1990). Jacobs et al. (2019) added a modified technique for KED to their protocol (KED+) using a 2-phase process, where the bird's head was extended at a 90° angle after the KED was applied in order to cause more neck damage and potentially quicker death. All reflex measures were longer with both mechanical techniques (KED and KED+) compared to manual cervical dislocation where brain stem death occurred more quickly.

CD and ACD resulted in all chicks having successful spinal cord transection. However, the spinal cord was transected at a more caudal location in all chicks. Anatomic damage to the more cranial cervical spinal cord is possible with spinal cord concussion, neurogenic shock, and loss of consciousness in humans (Dumont et al., 2001; Harrop et al., 2001). More caudal cervical dislocations and spinal cord transections could be the reason for lack of any subdural (SDH) and parenchymal (PCH) hemorrhage in the brain tissues of the chicks in the current study. Previous studies suggested that shorter latencies to insensibility and irreversible loss of functions are associated with SDH and PCH in the brain (Erasmus et al., 2010; Bader et al., 2014). Thus, more caudal cervical dislocation and spinal cord transection may relate to the longer time to loss of consciousness found in our experiment. None of the chicks killed by aMCD experienced cervical dislocation, and this was associated with no spinal cord transection and lack of brain hemorrhages. Thus, we suggest that KED-model-S was unable to cause brain trauma and spinal cord transection in the chicks, and this could be the reason for observed longer latency to brain stem death and clinical death in the chicks killed by aMCD.

All chicks in the current study died without application of a secondary method of killing, and without evidence of brain trauma. CD and ACD resulted in subdural hemorrhage at the site of cervical dislocation indicating disruption of the blood vessels. This suggests that cerebral ischemia was a cause of death in the chicks killed by CD or ACD. In humans, damage to the cervical area of the spinal cord impaired the functioning of some respiratory muscles, resulting in hypoxia due to respiratory failure, ultimately causing death (Winslow and Rozovsky, 2003). With all testing methods, a large proportion of chicks exhibited gasping. Gasping may have resulted from hypoxia due to spinal cord trauma, which may also have caused death in chicks killed by CD and ACD. However, chicks killed by MCD had no brain or spinal cord trauma and minimal SCH in the cervical area. Cartner et al. (2007) studied electroencephalograms (**EEG**) and visual evoked potentials (**VEP**) in mice after euthanasia by cervical dislocation and reported that 9.5% (2 of 21) of the mice who did not have luxation of the atlanto-occipital joint as documented by radiographs demonstrated declines in EEG and VEP measures similar to those who exhibited luxations.

The death of chicks was suggested to have happened due to cerebral ischemia and hypoxia as brain trauma was absent; explained by cervical dislocation that occurred more caudally in the cervical vertebrae in the chicks killed either by manual CD or assisted manual CD. Assisted manual CD appears to be more acceptable in commercial conditions due to the absence of external bleeding.

CONCLUSIONS

There was no difference in the efficacy of manual CD compared to assisted manual CD in layer chicks. Overall, mechanical cervical dislocation by KED-model-S resulted in a lower efficacy in comparison to manual and assisted manual cervical dislocation. KED-model-S cannot be recommended as a humane and efficient on-farm killing method for layer chicks due to prolonged latency to brain stem death and lower anatomical damage. The anesthetic protocol eliminated clonic convulsions and tonic convulsions in the current study, suggesting that these behavioral responses are no longer valid as an appropriate measure of brain death in cases where ketamine and medetomidine have been administered in chicks.

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DISCLOSURES

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REFERENCES

Alkire, M. T., A. G. Hudetz, and G. Tononi. 2008. Review: consciousness and anesthesia. Science 322:876–880.

American Veterinary Medical Association. 2020. AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. American Veterinary Medical Association, Schaumburg, IL. https://wwwavma.org/KB/Policies/Documents/euthanasia.pdf (accessed Dec. 2021).

Bader, S., B. Meyer-Kühling, R. Günther, A. Breithaupt, S. Rautenschlein, and A. D. Gruber. 2014. Anatomical and histologic pathology induced by cervical dislocation following blunt head trauma for on-farm euthanasia of poultry. J. Appl. Poult. Res. 23:546–556.

Baker-Cook, B., S. S. Torrey, T. M. Widowski, P. V. Turner, T. Knezacek, J. Nicholds, and K. Schwean-Lardner. 2021. The efficacy of three on-farm euthanasia methods for broiler chickens throughout the production cycle. Br. Poult. Sci. 62:638–649.

Bandara, R. M. A. S., S. Torrey, P. V. Turner, A. Linden, A. Bolinder, K. Schwean-Lardner, and T. M. Widowski. 2019a. Efficacy of a novel mechanical cervical dislocation device in comparison to manual cervical dislocation in layer chickens. Animals 9:407.

Bandara, R. M. A . S., S. Torrey, P. V. Turner, K. Schwean-Lardner, and T. M. Widowski. 2019b. Anatomical pathology, behavioral, and physiological responses induced by application of non-penetrating captive bolt devices in layer chickens. Front. Vet. Sci. 6:89.

Carbone, L., E. T. Carbone, E. M. Yi, D. B. Bauer, K. A. Lindstrom, J. M. Parker, J. A. Austin, Y. Seo, A. D. Gandhi, and J. D. Wilkerson. 2012. Assessing cervical dislocation as a humane euthanasia method in mice. J. Am. Assoc. Lab. Anim. Sci 15:352–356 51.

Cartner, S. C., S. C. Barlow, and T. J. Ness. 2007. Loss of cortical function in mice after decapitation, cervical dislocation, potassium chloride injection, and CO_2 inhalation. Comp. Med 1:570–573 57.

Dumont, R. J., D. O. Okonkwo, S. Verma, R. J. Hurlbert, P. T. Boulos, D. B. Ellegala, and A. S. Dumont. 2001. Acute spinal cord injury, part I: pathophysiologic mechanisms. Clin. Neuropharmacol. 24:254–264.

Erasmus, M. A., P. V. Turner, S. G. Nykamp, and T. M. Widowski. 2010. Brain and skull lesions resulting from use of percussive bolt, cervical dislocation by stretching, cervical dislocation by crushing and blunt trauma. Vet. Rec. 167:850–858.

Gregory, N. G., and S. B. Wotton. 1990. Comparison of neck dislocation and percussion of the head on visual evoked responses in the chicken's brain. Vet. Rec. 126:570–572.

Harrop, J., A. Sharan, A. R. Vaccaro, and G. J. Przybylski. 2001. The cause of neurologic deterioration after acute cervical spinal cord injury. Spine 26:340–346.

Hernandez, E., F. James, S. Torrey, T. Widowski, K. Schwean-Lardner, G. Monteith, and P. V. Turner. 2019. Evaluation of brain death in laying hens during on-farm killing by cervical dislocation methods or pentobarbital sodium injection. Front. Vet. Sci. 6:297.

Jacobs, L., D. V. Bourassa, C. E. Harris, and R. J. Buhr. 2019. Euthanasia: manual versus mechanical cervical dislocation for broilers. Animals 1:47.

Koechner MFG. Co., Inc. Tipton, MO (2016). http://www.turkeycoops.com/euthanizingdevice.html (Accessed Feb. 2019).

Martin, J. E., D. E. F. McKeegan, J. Sparrey, and V. Sandilands. 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. Anim. Welf. 25:227–241.

Martin, J. E., D. E. F. McKeegan, J. Sparrey, and V. Sandilands. 2017. Evaluation of potential killing performance of novel percussive and cervical dislocation tools in chicken cadavers. Br. Poult. Sci. 58:216–223.

Martin, J. E., D. E. Sandercock, V. Sandilands, J. Sparrey, L. Baker, N. H. C. Sparks, and D. E. F. McKeegan. 2018. Welfare risks of repeated application of on-farm killing methods for poultry. Animals 8:39.

Paul-Murphy, J., and J. Fialkowski. 2001. Injectable anesthesia and analgesia of birds. Recent Advances in Veterinary Anesthesia and Analgesia: Companion Animals. R. D. Gleed and J. W. Ludders, eds. International Veterinary Information Service, Ithaca, NY.

Poultry Industry Council. 2016. Practical guidelines for on-farm euthanasia of poultry. https://www.poultryindustrycouncil.ca/downloads/practical-guidelines-for-on-farm-euthanasia-of-poultry.pdf (accessed Feb. 2021).

Sandercock, D. A., A. Auckburally, D. Flaherty, V. Sandilands, and D. E. F. McKeegan. 2014. Avian reflex and electroencephalogram responses in different states of consciousness. Physiol. Behav. 133:252–259.

Winslow, C., and J. Rozovsky. 2003. Effect of spinal cord injury on the respiratory system. Am. J. Phys. Med. Rehabil. 82:803–814.

Woolcott, C. R., S. Torrey, P. V. Turner, H. Chalmers, L. Levison, K. Schwean-Lardner, and T. M. Widowski. 2018. Assessing a method of mechanical cervical dislocation as a humane option for on-farm killing using anesthetized poults and young turkeys. Front. Vet. Sci. 5:275.