Using time to insensibility and estimated time of death to evaluate a nonpenetrating captive bolt, cervical dislocation, and blunt trauma for on-farm killing of turkeys

M. A. Erasmus,* P. Lawlis,† I. J. H. Duncan,* and T. M. Widowski*[1]

*Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada, N1G 2W1; and †Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, Ontario, Canada, N1G 4Y2

ABSTRACT The effectiveness of a pneumatic nonpenetrating captive bolt (Zephyr) was assessed for on-farm euthanasia of turkeys and compared with blunt force trauma, manual cervical dislocation, and mechanical cervical dislocation using a burdizzo. The Zephyr (n = 46) and burdizzo (n = 26) were evaluated in turkey hens (11.4 ± 0.1 kg), the Zephyr (n = 46) and blunt trauma (n = 32) were evaluated in turkey toms (13.1 ± 0.2 kg), and the Zephyr (n = 12), blunt trauma (n = 11), and manual cervical dislocation (n = 7) were evaluated in broiler turkeys (4.1 ± 0.3 kg). The nictitating membrane and pupillary light reflexes were monitored continuously to determine when insensibility occurred. Time of death was estimated based on the end time of convulsions and sustained absence of breathing. The nictitating membrane reflex was present immediately after treatment in all 26 hens killed with a burdizzo versus 8 of 46 hens killed with the Zephyr ($P < 0.001$). The presence of eye reflexes did not differ between the Zephyr and blunt trauma for toms (1 of 26 toms killed with blunt trauma, 2 of 44 toms killed with the Zephyr, $P = 1.0$). The nictitating membrane reflex persisted in a greater proportion of broiler turkeys killed with cervical dislocation (7 of 7) versus the Zephyr (0 of 12, $P < 0.001$) and blunt trauma (2 of 9, $P = 0.003$) but did not differ between blunt trauma and the Zephyr ($P = 0.2$). End time of convulsions did not differ between the Zephyr and burdizzo for hens (204 ± 8 vs. 114 ± 10 s, $P = 0.5$) or between the Zephyr and blunt trauma for toms (200 ± 7 s vs. 218 ± 11.8 s, $P = 0.4$) but was shorter after cervical dislocation in broiler turkeys (cervical dislocation: 138 ± 13 s, Zephyr: 165 ± 7 s, blunt trauma: 178 ± 13 s, $P < 0.001$). Results demonstrated that the Zephyr (discharged twice in immediate succession) and blunt trauma (single hit) were similarly effective at consistently causing immediate insensibility. Conversely, neither method of cervical dislocation caused immediate insensibility. This study may assist in revising current poultry euthanasia recommendations.

Key words: on-farm killing, turkey, blunt trauma, cervical dislocation, nonpenetrating captive bolt

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INTRODUCTION

Over 20 million turkeys are produced annually in Canada (Statistics Canada, 2009), and 250 million are produced annually in the United States (USDA, 2009). As with all animal production systems, injury and disease inevitably occur, rendering some animals unfit for shipping and slaughter. Under these circumstances, on-farm euthanasia is necessary to reduce suffering. Euthanasia or "good death" requires that death occurs with minimal pain and distress, and that loss of consciousness is rapid, followed by irreversible impairment of brain function and respiratory or cardiac arrest (AVMA, 2007).

To evaluate whether any particular on-farm killing method can be considered effective for euthanasia, it is necessary to determine when the method results in complete loss of consciousness (insensibility) and if insensibility is followed by irreversible loss of respiration and brain and heart function. Electroencephalography is arguably the most reliable measure of insensibility and brain death because it monitors activity in the cerebral cortex, which is necessary for consciousness. Similarly, evoked responses involve use of the electroencephalograph (**EEG**) to determine whether there is brain activity in response to external stimuli. However, use of the EEG and evoked responses is limited to the laboratory and cannot practically be used in the field to assess insensibility and death after on-farm killing. Moreover, EEG electrodes may be destroyed or damaged when killing methods are used that cause direct damage to the head and brain.

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[1]Corresponding author: twidowsk@uoguelph.ca

In humans, brainstem reflexes are important clinical determinants of brain death, which is generally defined as irreversible loss of brain function, including the brainstem (Wijdicks, 1995). Similarly, the absence of brainstem reflexes, such as the pupillary light and corneal reflexes, are indicative of loss of brain function in animals after stunning and slaughter and are more practical for assessing insensibility and brain death in the field. The pupillary light reflex is a reliable indicator of complete insensibility (Croft, 1961) and is present when there is pupil constriction in response to light shone in the eye. The corneal reflex can easily be observed as blinking or movement of the nictitating membrane and has been used to assess insensibility in other studies of killing methods for birds (Wotton and Sparrey, 2002). Absence of the corneal reflex in addition to a fixed, dilated pupil indicates that there is reduced blood flow to the brainstem (Heard, 2000), which leads to brain death if blood flow is not restored.

Other techniques that have been used in scientific assessments of insensibility after stunning or killing include behavioral indicators, such as the absence of neck tension, which has been used as an indicator of insensibility with gas killing methods (Mohan Raj et al., 1992; Lambooij et al., 1999) and captive bolt shooting (Raj and O'Callaghan, 2001) and has been shown to occur at the same time as suppression of brain activity measured with the EEG (Gerritzen et al., 2004). Jaw tone is another less common method that may be used to assess insensibility during stunning and killing because it is used to evaluate insensibility during avian anesthesia (Heard, 2000).

Cessation of convulsions has been evaluated for practically assessing time of brain failure in field conditions (Dawson et al., 2007, 2009). Convulsions are typical reflex reactions that occur in animals after stunning with physical methods (Blackmore and Delany, 1987) and also occur after decapitation (Eichbaum et al., 1975) and during anoxia (Gildea and Cobb, 1930; Lopes da Silva, 1983). In birds, convulsive activity includes a clonic phase, which is characterized by one or more episodes of violent wing-flapping. This is followed by a tonic phase, which is characterized by rigid muscular contraction of the legs. Convulsive activity has been shown to cease about 12 s before EEG silence (Dawson et al., 2009).

In Canada, recommended euthanasia methods for routine culling include cervical dislocation for young turkeys and blunt force trauma, delivered as a sharp firm blow to the head with a blunt instrument, for older turkeys (CARC, 2003). These methods are recommended in the United States as well, in addition to CO_2 and captive bolt pistols (NTF, 2004). Cervical dislocation can be applied manually, which involves stretching and separating the vertebrae by hand or mechanically, which involves the use of a tool such as bovine burdizzo castration forceps (burdizzo) to sever or crush the vertebrae (Galvin et al., 2005). Manual cervical dislocation is acceptable for growing turkeys (NTF, 2004) but may not be effective for mature and market age turkeys (NTF, 2004; CCAC, 2009). Hence, mechanical cervical dislocation is sometimes recommended for the euthanasia of large birds when manual means are difficult to apply (CFIA, 2007; Saif, 2008; CCAC, 2009). Both manual and mechanical cervical dislocation are listed as killing methods for poultry by the World Organization for Animal Health for the purposes of disease control (Galvin et al., 2005). Although cervical dislocation and blunt trauma are the methods most commonly used on commercial turkey farms and are thought to be humane, there is little scientific evidence to confirm this observation (AVMA, 2007). Evidence for the effectiveness of blunt trauma is particularly scarce, and previous research with chickens has shown that cervical dislocation does not consistently result in immediate insensibility (Gregory and Wotton, 1990).

Gregory and Wotton (1990) used visual evoked responses to assess the effectiveness of cervical dislocation and percussive stunning using a nonpenetrating captive bolt pistol in 40 broiler chickens with a mean (±SD) weight of 2.3 ± 0.3 kg. Visual evoked responses were present in all 24 chickens after cervical dislocation, whereas complete loss of visual evoked responses occurred in 3 of 8 chickens stunned with the nonpenetrating captive bolt, and evoked responses were severely reduced in the remaining 5 birds (Gregory and Wotton, 1990). Visual evoked responses returned if birds that were stunned with the nonpenetrating captive bolt were ventilated with air (Gregory and Wotton, 1990), indicating that the nonpenetrating captive bolt may stun birds but not result in death. Because the purpose of that study was only to compare the immediate effects of each treatment, the authors did not discuss how overall effects and time to death differed between cervical dislocation and the nonpenetrating captive bolt. Hence, cervical dislocation appears to be variable in its effectiveness for causing immediate insensibility in broiler chickens, and there is no published literature on its effects in turkeys. In addition, further research is necessary to examine whether a nonpenetrating captive bolt consistently results in death in poultry.

The objectives of this study were to evaluate the effectiveness of a pneumatic nonpenetrating captive bolt for euthanasia of turkeys in 3 weight categories (broilers, hens, and toms) using brainstem reflexes as indicators of insensibility and the cessation of convulsions and sustained absence of breathing as indicators of irreversible brain failure and death. In addition, we sought to compare the captive bolt with methods currently used for on-farm killing of turkeys: manual and mechanical cervical dislocation and blunt trauma.

MATERIALS AND METHODS

Ethical Note

All methods were approved by the University of Guelph Animal Care Committee under guidelines of

the Canadian Council on Animal Care. Only birds that were already scheduled for culling were used in these experiments. In the case of hens, data were collected when a research project concluded and birds were killed for sample collection using a burdizzo, which is the approved method at that research facility. Farms producing the different weight classes of turkeys were contacted to determine their willingness to participate in the study. Cull birds were usually identified at the end of a production cycle when the birds were being shipped for slaughter. Cull birds on farms were designated for killing based on conditions such as injury or infections that prevented them from being shipped for slaughter. On any given day, birds that had been identified for killing were randomly allocated to treatment groups (killing methods). The birds were removed from the barn and carried to a separate room or hallway for killing and data collection. Killing procedures were carried out by stock people on each farm whose normal duties included humane killing.

Equipment

The nonpenetrating captive bolt pistol (Zephyr) used in these experiments was initially developed and approved by the Ontario Ministry of Agriculture, Food and Rural Affairs for stunning rabbits in abattoirs. The Zephyr consisted of a pneumatic nail gun (NS 100A 1/4" Narrow Crown Stapler, Porter Cable, Jackson, TN) that was fitted with a convex nylon head (diameter: 25 mm, length: 38 mm) attached to a cylindrical metal bolt (diameter: 8 mm). The nylon head was recessed 33 mm from the end of the gun barrel. When the bolt was fully extended, the nylon head protruded 17 mm past the gun barrel and could travel a maximum distance of 50 mm. A nail gun that had been modified into a penetrating captive bolt was first tested by Raj and O'Callaghan (2001), who found that stunning and death resulted in broiler chickens (mean ± SD, 2.9 ± 0.2 kg) when a steel bolt with a diameter of at least 6 mm and a penetration depth of 10 mm was applied perpendicularly to the top of the head at an airline pressure of 827 kPa.

Figure 1. Placement of the Zephyr. The bird should be restrained in sternal recumbency with its neck resting ventrally on the ground. The Zephyr should be held perpendicular to the frontal bone, gently resting on the bird's head. The proper location for causing immediate insensibility is on the midline between the ears and the eyes, directly above the cerebral cortex, where the skull is thinnest and where there is a natural depression in the skull.

Initial pilot studies on turkey carcasses determined that the Zephyr should be applied perpendicularly to the frontal bone on the midline between the ears and the eyes, directly above the cerebral cortex (Figure 1). Postmortem examinations indicated that the skull was thinnest at this location, providing the least amount of protection to the brain. Furthermore, consciousness depends on the integrity of the cerebral cortex (Strich, 1956; Brierley et al., 1971); therefore, insensibility would likely be best achieved when discharging the Zephyr directly above the cortex. In an on-farm pilot study with 6 cull birds, application of the Zephyr using an airline pressure of 827 kPa resulted in immediate insensibility leading to death.

Birds and Facilities

The effectiveness of the Zephyr was evaluated in turkey hens, turkey toms, and broiler turkeys and compared with blunt trauma and cervical dislocation in 3 experiments. In all experiments, some form of restraint was necessary because physical killing methods result in severe wing-flapping, making it difficult to take measurements. Once wing-flapping had ceased, birds were freed from restraint and rolled onto their backs so that the end time of tonic convulsions and absence or return of breathing could be fully examined.

Experiment 1. Data were collected for 72 commercial white turkey hens at approximately 94 wk of age (11.4 ± 0.1 kg) that were part of a separate study at a research facility that routinely uses bovine burdizzo castration forceps (castrator for bulls KVS 376-504, Kane Veterinary Supplies Ltd., Cambridge, Ontario, Canada) for mechanical cervical dislocation of mature chickens and turkeys. In total, 46 hens were killed with the Zephyr by 3 stock people and 26 hens were killed using the burdizzo by 5 other stock people. Hens were restrained in large metal bleeding cones. For hens that were killed with the burdizzo, cones were placed upside down in a metal stand designed specifically for holding the cones. All hens that were killed with the Zephyr were restrained in metal cones placed horizontally on the ground because this enabled sternal recumbency with the head on a hard or concrete surface and proper placement of the Zephyr. The Zephyr was discharged at an airline pressure of 758 to 827 kPa. If reflexes were present after discharging the Zephyr, it was discharged a second time.

Experiment 2. Data were collected on 2 commercial turkey farms where the routine killing method consisted of blunt force trauma using a metal pipe (farm A) or metal bat (farm B). A third farm routinely used a burdizzo-style instrument. However, observations on 4 turkeys indicated that responses were similar to those obtained from hens killed with the burdizzo. Data collection was terminated and the Zephyr was subsequently applied to these 4 toms to render them insensible. For ethical reasons, and in accordance with our animal care approval, remaining birds were all killed with the Zephyr.

Overall, data were collected for 78 turkey toms between 17 and 19 wk of age (13.1 ± 0.2 kg); 46 toms were killed with the Zephyr and 32 were killed with blunt trauma. On each farm, 2 stock people whose normal duties included humane killing performed the killing methods. To use the Zephyr, stock people were instructed to gently hold the bird behind its head, place the head so that it rested flat on a hard surface, and then discharge the Zephyr twice in immediate succession using an airline pressure of 794 to 827 kPa. If reflexes persisted after the double application, the Zephyr was immediately discharged again. This was considered a misstun and data collection was terminated in these cases. Blunt trauma was delivered perpendicularly to the top of the head with the stock person swinging the bat or pipe in a downward direction. All toms were restrained in fabric jackets similar to the jacket designed by Evans and Kear (1972), but jackets were removed once clonic convulsions ceased.

Experiment 3. A total of 30 broiler turkeys were killed at a commercial farm using the Zephyr, blunt trauma, or manual cervical dislocation. The Zephyr was administered to 12 broiler turkeys at 7 wk of age (4.6 ± 0.3 kg) using an airline pressure of 724 to 827 kPa, and if reflexes persisted after the double application, the Zephyr was immediately applied again. Broilers killed with the Zephyr were restrained in fabric jackets based on those designed by Evans and Kear (1972), but jackets were reduced in length by 28 cm and reduced in width by 4 cm to accommodate the smaller birds.

An experienced stock person performed manual cervical dislocation on 7 broilers at 3 wk of age (1.6 ± 0.1 kg). All turkeys killed with manual cervical dislocation weighed less than 3 kg, as recommended in the Animal Welfare Guide to On-Farm Stunning and Euthanasia of Specialty Poultry and Barnyard Fowl published by the Quebec Ministère de l'Agriculture, des Pêcheries et de l'Alimentation (MAPAQ, 2008). After restraining the bird in the jacket, cervical dislocation was applied by holding the bird by the shanks and then grasping the head behind the skull with 2 fingers and quickly and firmly pulling the head down and against the knuckle of the first finger to stretch the neck and separate the vertebrae. After cervical dislocation, the birds were placed on the ground to examine reflexes.

Another experienced stock person administered blunt trauma to 11 broilers at 7 wk of age (5.0 ± 0.3 kg) by swinging a metal pipe straight down and delivering the blow perpendicularly to the top of the head. If reflexes persisted after the first blow, a second blow was delivered. This was considered a misstun and data collection was terminated in these cases. All broilers except for the first 2 birds that were killed with blunt trauma were not restrained because the smaller birds, being restrained, presented a much lower target and the stock person felt that it would reduce the effectiveness of the blow. Therefore, 9 of 11 broilers were left unrestrained on the ground. Although these broiler turkeys were not restrained, reflexes could still be assessed, and one person effectively restrained the broilers during clonic convulsions while another person examined the reflexes.

Data Collection

In all experiments, brainstem reflexes were chosen as the primary means of assessing insensibility. The nictitating membrane reflex and pupil constriction, which was examined using a standard white light-emitting diode penlight (KM200GST06A Nichia penlight, Garrity Industries Canada Ltd., Mississauga, Ontario, Canada), were tested immediately after administration of the killing method. These reflexes were monitored continuously until they disappeared to determine if and when insensibility occurred (Table 1). The occurrence and duration of gasping and end time of convulsions were also recorded (Table 1). Because there may be a few episodes of wing-flapping during clonic convulsions, the time at which all motion ceased and the birds relaxed, rather than the absolute duration of convulsions, was recorded. The end time of tonic convulsions and the sustained absence of breathing were used to judge when brain death occurred.

In some cases, it was not possible to test eye reflexes due to conditions affecting the eyes; therefore, neck muscle tension and jaw tone were observed as alternative measures of insensibility. Jaw tone was tested by pushing the lower jaw down and examining whether there was any resistance to the downward pressure. Resistance indicated that jaw tone was present. Similarly, neck muscle tension was assessed by lightly lifting the neck and examining whether there was resistance.

Statistical Analysis

All statistical analyses were computed in SAS (SAS Inst. Inc., Cary, NC). Fisher's exact tests on 2 × 2 contingency tables were used to test the null hypothesis that the proportions of birds in which reflexes and gasping were present immediately after treatment and the proportion of birds requiring a repeated treatment in experiments 2 and 3 were independent of killing method (Zar, 1998). Fisher's exact test on 2 × 2 contingency tables was also used to test the null hypothesis that the proportion of turkey hens in experiment 1 that

Table 1. Measures tested immediately after treatment to assess insensibility[1]

Measure	Description	Procedure
Nictitating membrane reflex	Third eyelid that moves over the cornea	The medial canthus of the eye or the cornea was lightly touched with a fingertip
Pupillary light reflex (pupil constriction)	Constriction of the pupil in response to light	A standard penlight was shone into the eye and pupil constriction was examined
Convulsions	Clonic: episodes of wing-flapping Tonic: becoming rigid and showing final paddling motions with the legs and wings stretched (Raj et al., 1990)	The time of cessation of all movement was recorded Convulsions ceased when the limbs were relaxed
Gasping	Deep breathing with the open mouth (Gerritzen et al., 2004)	
Jaw tone	Resistance in response to downward pressure applied to the jaw	Gentle pressure was applied to the lower jaw with a finger
Neck muscle tension	Change in neck muscle tone or movement of the head when the neck is lifted	The neck was lifted with the fingers of one hand

[1]Absence of reflexes indicated a state of insensibility.

required a repeated treatment due to the persistence of reflexes was independent of stock person.

In all 3 experiments, the effect of killing method on the end time of convulsions was examined. For experiment 1, a mixed model was used to compare the effects of killing method (Zephyr and burdizzo) on the log-transformed end time of convulsions. Killing method was included as a fixed effect, and end time of convulsions (log-transformed) was included as the dependent variable. Stock person was included as a random effect nested within killing method, and BW was included as a covariate. A mixed model was used to compare the effects of killing method (Zephyr and blunt trauma) on the log-transformed end time of convulsions for experiment 2. The experiment was conducted on 3 different farms; therefore, farm was included as a blocking variable. Body weight was included as a covariate and stock person was included as a random effect nested within farm. In experiment 3, four different stock people at a single commercial facility applied treatments; 1 person each applied cervical dislocation and blunt trauma, and 2 people applied the Zephyr. A GLM that included the 4 treatment-stock person combinations as factors and that included BW as a covariate was used to compare the log-transformed end time of convulsions among the 3 killing methods. Data from all 3 experiments are presented in a single table for simple comparison (Table 2). Statistical significance was defined as $P < 0.05$.

RESULTS

Experiment 1

Presence of the nictitating membrane reflex and gasping after treatment differed between the burdizzo and the Zephyr (Table 3). The nictitating membrane reflex was present and gasping occurred in all hens after application of the burdizzo, compared with 17% of hens that had reflexes present and 7% of hens that were gasping when the Zephyr was used ($P < 0.001$).

The nictitating membrane was relatively easy to observe, even during severe wing-flapping, whereas assessment of the pupillary light reflex required the bird's head to be kept still until absence of pupil constriction could be verified. In addition, lighting conditions needed to be optimal to assess pupil constriction. Due to severe wing-flapping and suboptimal lighting conditions, the pupillary light reflex could only be reliably tested in 16 hens killed with the burdizzo and 38 hens killed with the Zephyr. The pupillary light reflex was present in all 16 hens killed with the burdizzo and in 1 hen killed with the Zephyr immediately after treatment (P

Table 2. Mean end time of convulsions and duration of the nictitating membrane reflex (s) for turkey hens (experiment 1), turkey toms (experiment 2), and broiler turkeys (experiment 3) killed with the Zephyr, burdizzo, cervical dislocation, and blunt trauma

Weight class	Killing method	Mean (±SE) nictitating membrane reflex duration (s)	Mean (±SE) convulsions end time (s)
Hen	Burdizzo	106 ± 7	114 ± 10
	Zephyr	0[1]	204 ± 8
Tom	Blunt trauma	0[1]	218 ± 12
	Zephyr	0[1]	200 ± 7
Broiler	Blunt trauma	0[1]	178 ± 13[a]
	Cervical dislocation	43 ± 11	138 ± 13[b]
	Zephyr	0[1]	165 ± 7[a]

[a,b]Means within columns for a given weight class lacking common superscripts differ ($P < 0.05$).

[1]If reflexes were present after the Zephyr and blunt trauma were applied, birds were immediately hit a second time to completely abolish reflexes.

Table 3. The number of turkey hens in each treatment group in experiment 1 in which the nictitating membrane reflex and gasping were observed immediately after a single application of the Zephyr and application of the burdizzo

Method	n	Nictitating membrane reflex present (n)	Gasping present (n)
Zephyr	46	8^{B}	3^{B}
Burdizzo	26	26^{A}	26^{A}

^{A,B}Frequencies within columns lacking common superscripts differ from random expectation ($P < 0.01$) when using Fisher's exact test (df = 1).

< 0.001). However, the pupillary light reflex could not be verified in the other 7 hens killed with the Zephyr in which the nictitating membrane reflex was present.

The nictitating membrane reflex persisted on average 106 ± 11 s, the pupillary light reflex persisted on average 119 ± 15 s, and gasping lasted on average 109 ± 15 s in hens killed with the burdizzo.

Convulsions occurred in all hens after both treatments. The mean end time of convulsions (Table 2) did not differ between treatments (Zephyr: 203 ± 8 s, cervical crushing: 114 ± 10 s, $F = 0.4$, $P = 0.5$). Convulsions started immediately in all birds killed with the burdizzo and all but 3 birds killed with the Zephyr. In those 3 birds, convulsions started at 2, 7, and 8 s after treatment, respectively.

The nictitating membrane reflex returned in 1 hen (2%) 1 min after the Zephyr was applied but was abolished when the Zephyr was immediately applied again. The proportion of birds that had to be treated a second time due to the persistence of reflexes was highest for stock person C (Table 4). The person applying the Zephyr may have been a factor affecting the proportion of birds that had to be treated a second time due to improper stunning and killing. Therefore, the Zephyr was applied twice in immediate succession, instead of using a single application, in subsequent experiments with toms and broilers.

Experiment 2

Eye reflexes were assessed in 26 (81%) turkey toms killed with blunt trauma and 43 (93%) turkey toms killed with the Zephyr. Eye reflexes could not be examined in 6 toms killed with blunt trauma because blunt trauma resulted in damage to the eyes in 4 toms, causing proptosis and hemorrhage in the eyes. In 2 other toms, the eyes were swollen due to irritation or infection before treatment, preventing the eye reflexes from being examined. In 5 other toms killed with blunt trauma, one of the eyes was damaged due to blunt trauma, but reflexes were tested in the unaffected eye and have been included in the results. Reflexes could not be examined in 2 toms killed with the Zephyr due to eye irritation or infection before treatment. In 3 other toms killed with the Zephyr, one of the eyes was affected by infection or irritation, but reflexes were tested in the unaffected eye and have been included. For birds in which eye reflexes could not be tested, gasping, neck muscle tension, and jaw tone were examined, and all of these measures were absent in both treatment groups.

Eye reflexes were present immediately after treatment in 2 turkey toms (3%) killed with the Zephyr and 1 tom (4%) killed with blunt trauma ($P = 1.0$). All toms were observed for gasping, but gasping only occurred in 1 tom in which reflexes were present immediately after treatment with the Zephyr (n = 46), and no gasping was observed in toms killed with blunt trauma (n = 32, $P = 1.0$). Results for each farm are presented in Table 5.

Convulsions occurred in toms in both treatment groups after treatment, including toms in which reflexes were still present after treatment. The end time of convulsions (Table 2) was recorded for 31 (97%) toms killed with blunt trauma and all 46 toms killed with the Zephyr, and mean duration did not differ between treatments (Zephyr: 200 ± 7 s, blunt trauma: 218 ± 11 s, $F = 1.7$, $P = 0.18$). Convulsions started immediately after treatment in all toms killed with blunt trauma. Convulsions started at 2, 4, 11, and 14 s after treatment in 4 toms killed with the Zephyr but started immediately after treatment in all other toms killed with the Zephyr. Rhythmic breathing returned in 1 tom in the Zephyr group 3 min after the Zephyr was applied, and reflexes returned in 1 tom in the blunt trauma group 1 min after blunt trauma was administered; both toms were immediately struck a second time. After the second strike, breathing ceased and reflexes were abolished permanently. Overall, the proportion of toms that had to be treated again due to the persistence and return of reflexes did not differ between the Zephyr and blunt trauma (Zephyr = 3, blunt trauma = 2, $P = 1.0$).

Experiment 3

Eye reflexes were assessed in all 12 broiler turkeys killed with the Zephyr but could only be assessed in 9 of 11 broiler turkeys killed with blunt trauma. Eye reflexes could not be tested in those 2 broiler turkeys killed with blunt trauma due to eye proptosis and hemorrhage. Reflexes could only be tested in 1 eye for 3 broiler turkeys killed with blunt trauma and 3 broiler turkeys killed with the Zephyr due to proptosis of the

Table 4. The number of turkey hens in experiment 1 that each stock person treated with the Zephyr and the number of those hens that each stock person had to immediately treat again because of the persistence of reflexes

Person	Birds treated (n)	Misstunned (n)
A	7	3^{ab}
B	29	3^{b}
C	10	5^{a}

^{a,b}Frequencies within columns lacking common superscripts differ from random expectation ($P < 0.05$) when using Fisher's exact test based on 2 × 2 contingency tables (df = 1).

Table 5. The number of turkey toms on each farm in experiment 2 in which reflexes and gasping were observed immediately after application of the Zephyr (2 shots applied in immediate succession) or blunt trauma and in which reflexes or breathing returned

Farm	Method	n	Reflexes present (n)	Return of reflexes or breathing (n)
A	Zephyr	10	1	0
	Blunt trauma	11	0	1
B	Zephyr	10	0	0
	Blunt trauma	15	1	0
C	Zephyr	24	1	1

other eye. Broiler turkeys in which reflexes could only be tested in 1 eye have been included in the results.

Pupil constriction could not be reliably determined for 4 broiler turkeys killed with manual cervical dislocation because experiments were conducted in bright light, making it difficult to accurately examine pupil constriction. The nictitating membrane reflex was assessed in all 7 broiler turkeys.

No jaw tone, neck muscle tension, or gasping was observed immediately after treatment in broiler turkeys killed with the Zephyr or blunt trauma in which eye reflexes could not be tested. The nictitating membrane reflex was present in all broiler turkeys killed with cervical dislocation, compared with 2 broiler turkeys killed with blunt trauma, and none of the broiler turkeys killed with the Zephyr (Table 6). The proportion of broiler turkeys in which the nictitating membrane was present after treatment was greater with cervical dislocation than with blunt trauma ($P = 0.003$) and with the Zephyr ($P < 0.001$) but did not differ between the Zephyr and blunt trauma ($P = 0.2$). Similarly, the occurrence of gasping (Table 6) was greatest for broiler turkeys killed with cervical dislocation compared with blunt trauma ($P = 0.04$) and the Zephyr ($P = 0.003$) but did not differ between the Zephyr and blunt trauma ($P = 0.2$).

Pupil constriction was present in all 3 broiler turkeys killed with cervical dislocation in which pupil constriction could be assessed. Pupil constriction and gasping were also present in the 2 broiler turkeys killed with blunt trauma in which the nictitating membrane reflex was present.

In broiler turkeys killed with cervical dislocation, the nictitating membrane reflex persisted on average 43 ± 7 s. The duration of gasping was recorded for 3 of 6 broilers killed with cervical dislocation and lasted on average 39 ± 3 s. In 1 broiler turkey, cervical dislocation resulted in partial removal of the head from the neck and blood loss. The shortest nictitating membrane reflex duration (15 s) was noted in this turkey, and no gasping was observed.

Convulsions occurred in all broiler turkeys regardless of the killing method used. End time of convulsions did not differ between the 2 stock people who applied the Zephyr ($P = 0.2$); results for the Zephyr were pooled for further comparison among treatments. The end time of convulsions was obtained for all broiler turkeys (Table 2) and mean end time was longer on average with the Zephyr and blunt trauma than with cervical dislocation (Zephyr: 165 ± 7 s, blunt trauma: 178 ± 13 s, cervical dislocation: 138 ± 13 s, $F = 49.8$, $P < 0.001$). Convulsions started immediately in all broiler turkeys killed with manual cervical dislocation and blunt trauma and in all but 2 broilers killed with the Zephyr. Convulsions started at 5 and 16 s, respectively, after treatment in those 2 broilers.

Reflexes returned and gasping occurred 30 s after blunt trauma in 1 broiler turkey but were permanently abolished with a second treatment. The number of turkeys that had to be treated a second time due to the persistence and return of reflexes did not differ between the blunt trauma and Zephyr treatment groups (Zephyr = 0, blunt trauma = 3, $P = 0.06$).

DISCUSSION

This is the first study to evaluate the effectiveness of a pneumatic nonpenetrating captive bolt, manual cervical dislocation, mechanical cervical dislocation, and blunt trauma for humane killing of turkeys. Results demonstrated that the Zephyr and blunt trauma consistently induced immediate insensibility leading to death, whereas all birds showed signs of sensibility after manual and mechanical cervical dislocation.

Table 6. The numbers of broiler turkeys in experiment 3 in which the nictitating membrane reflex was present and gasping occurred immediately after application of the Zephyr (discharged twice in immediate succession), blunt trauma, or manual cervical dislocation

Method	n	Nictitating membrane reflex present (n)	Gasping present (n)	Return of reflexes (n)
Zephyr	12	0^{b}	0^{b}	0
Blunt trauma	9	2^{b}	2^{b}	1
Cervical dislocation	7	7^{a}	6^{a}	0

[a,b]Frequencies within columns lacking common superscripts differ from random expectation ($P < 0.05$) when using Fisher's exact test based on 2 × 2 contingency tables (df = 1).

The first objective was to evaluate the Zephyr for humanely killing turkeys in 3 weight categories. Results from the present study complement those of previous research examining the effects of a nonpenetrating captive bolt on visual evoked responses in chickens (Gregory and Wotton, 1990). The Zephyr consistently caused immediate insensibility in turkey hens, turkey toms, and broiler turkeys. However, to effectively produce immediate insensibility, the Zephyr had to be applied directly above the cerebral cortex. Therefore, immediate insensibility resulted more consistently when the Zephyr was applied twice in immediate succession because this reduced the proportion of birds that had to be treated again due to the persistence of reflexes in experiment 2 (4%) compared with experiment 1 (17%).

Gregory and Wotton (1990) reported that visual evoked responses returned in simplified form if birds were ventilated with air, which is indicative of a return to sensibility. Under the field conditions of experiment 1, in which the Zephyr was only applied once unless reflexes persisted, reflexes returned in 1 hen. In experiment 2, rhythmic breathing returned in 1 tom when the Zephyr was applied twice in immediate succession, whereas no return of reflexes and breathing occurred in broiler turkeys killed with the Zephyr. Therefore, the size of the bird, location, and number of applications may have been factors influencing return to sensibility when the nonpenetrating captive bolt was used. The stock person's level of training may also have affected return to sensibility. Therefore, training should be provided if nonpenetrating captive bolts are considered for on-farm use.

The second objective of this study was to compare the effectiveness of the Zephyr to methods that are currently used for on-farm killing of turkeys. The Zephyr and blunt trauma were similarly effective at inducing insensibility leading to death in turkey toms. However, blunt trauma appeared to be more variable for smaller turkeys, as indicated by the greater proportion of broiler turkeys (22%) compared with turkey toms (3%) that had to be struck a second time due to the persistence of reflexes. This may have been due to the smaller targets presented by the smaller turkeys. The proportion of turkeys that regained sensibility was similar for the Zephyr and blunt trauma, with 1 tom in each treatment group regaining sensibility as indicated by the return of reflexes and rhythmic breathing. The instrument used for blunt trauma differed between farms (a metal pipe vs. a metal bat). There did not appear to be any differences between the effectiveness of the 2 instruments because only 2 toms of 32 killed with blunt trauma had to be struck a second time, and both toms were from different farms (reflexes persisted in 1 tom on farm B and reflexes returned in 1 tom on farm A).

The present results further complement those of Gregory and Wotton (1990), who also examined the effects of cervical dislocation by stretching and by crushing on visual evoked responses in chickens. Their results demonstrated that 25% of birds that were subjected to cervical dislocation by stretching and 69% of birds subjected to cervical dislocation by crushing did not show any immediate changes in their visual evoked responses, and none of those birds showed complete loss of visual evoked responses. Similarly, the present study found that eye reflexes persisted in all hens killed with cervical crushing using a burdizzo and all broilers killed with manual cervical dislocation. Gregory and Wotton (1990) concluded that cervical dislocation by stretching was more effective than cervical dislocation by crushing. Their conclusion was based on the observation that of 4 birds that experienced large reductions in the amplitude of visual evoked responses, 3 had been killed with cervical dislocation by stretching. In the present study, the duration of eye reflexes was shorter for broiler turkeys killed with cervical dislocation (43 ± 7 s) than for turkey hens killed with the burdizzo (106 ± 11 s). However, it cannot be concluded that manual cervical dislocation was better than mechanical cervical dislocation with the burdizzo because reflexes persisted in all turkeys in both groups after treatment.

When compared with other species, the results presented here are inconsistent with previously published results on the effects of cervical dislocation (Weisbrod et al., 1984; Iwarsson and Rehbinder, 1993). In mice, manual cervical dislocation resulted in rapid insensibility as measured using animal responses (Iwarsson and Rehbinder, 1993). However, the authors measured respiration and behavioral responses such as excitement, exploratory behavior, and urination and defecation and did not use brainstem reflexes or the EEG and evoked responses, which are more direct measures of brain activity and insensibility. More recent research using EEG telemetry and visual evoked responses demonstrated that cortical function in mice was significantly decreased within 5 and 10 s after cervical dislocation, indicating that rapid insensibility can be achieved in mice using this technique (Cartner et al., 2007). Cervical dislocation has also been noted to stop corneal reflexes and respiration immediately in rabbits (Weisbrod et al., 1984). Because the aforementioned studies did not all use the same measures of insensibility, it is difficult to draw conclusions regarding cervical dislocation across species. However, the use of brainstem reflexes and respiration in the present study and in rabbits (Weisbrod et al., 1984) and the use of EEG and evoked responses in mice (Cartner et al., 2007), suggest that there may be important species differences in the effectiveness of cervical dislocation.

Measures used to assess insensibility in the current study included brainstem reflexes [pupillary light and nictitating membrane (corneal) reflexes] and loss of jaw tone and neck muscle tension. The nictitating membrane reflex was relatively easy to observe in sensible birds in the field, whereas the pupillary light reflex proved to be more problematic due to severe convulsions after treatment and lighting conditions. Although the pupillary light reflex is the most reliable indicator of insensibility (Croft, 1961), the nictitating membrane

reflex may be more practical for assessing insensibility in the field.

In experiments 2 and 3, eye reflexes could not be tested in 7 turkey toms and 2 broiler turkeys killed with blunt trauma and 3 turkey toms killed with the Zephyr because the eyes were swollen in some birds due to eye irritation or infection before the experiments and in the other birds, blunt trauma caused damage to the eyes. Jaw tone and neck muscle tension were assessed instead and were absent in all of these birds immediately after treatment. Although the eye reflexes could not be assessed in those birds, the other measures indicated that birds were likely insensible immediately after treatment. Neck arching was observed during convulsions after blunt trauma and application of the Zephyr; however, this was probably not indicative of sensibility because it also occurred in turkeys in which reflexes were absent after treatment. Moreover, an arched neck, together with the head directed in a vertical position, is considered to be a sign of effective electrical stunning (DEFRA, 2007). Together, these results demonstrate that assessment of some reflexes may not work under all field conditions, and it is therefore important that the assessment of insensibility is not based on a single measure.

The irreversibility of insensibility and the time of brain death were determined using the time when all movement ceased together with the sustained absence of breathing. Some researchers have found that convulsions occur after the onset of insensibility (Mohan Raj et al., 1990, 1992). Results from the present study support the results of others, who found that convulsions occur before insensibility (Lambooij et al., 1999; Coenen et al., 2009). All turkeys in which reflexes persisted exhibited severe wing-flapping after treatment, supporting the idea that the onset of convulsions does not necessarily correspond with the onset of insensibility. However, as reported by Dawson et al. (2007, 2009), the cessation of movement can be used to estimate when irreversible brain death occurs, and together with the absence of breathing, this indicated when irreversible brain failure occurred in all turkeys in the present study.

When the end time of convulsions was compared between treatment groups for toms, there was no difference between the Zephyr and blunt trauma. Similarly, the end time of convulsions in turkey hens did not differ between the Zephyr and cervical crushing with the burdizzo. Conversely, the end time of convulsions in broilers occurred later with the Zephyr and blunt trauma than with the cervical dislocation. Cardiac arrest typically occurs after all motion has ceased (Dawson et al., 2007); therefore, it would appear that death occurred sooner with cervical dislocation than with the Zephyr and blunt trauma. However, reflexes persisted on average 43 ± 11 s with cervical dislocation, whereas reflexes were consistently abolished immediately after application of the Zephyr and blunt trauma. In terms of animal welfare, the time at which insensibility occurs is more important than the time at which death occurs because insensible animals are no longer able to feel pain and distress. Research is needed to further examine the relationship between the onset of insensibility and the time at which death occurs with different poultry killing methods.

Another difference between treatments was that gasping occurred in all turkeys killed with the burdizzo and manual cervical dislocation, and reflexes were present in all birds that were gasping, whereas no gasping was observed in birds effectively stunned and killed with the Zephyr and blunt trauma. Gasping is a reflex that is associated with autoresuscitation and occurs in response to hypoxia and depression of respiratory centers in the brainstem (Lumsden, 1923a,b). Therefore, cervical dislocation (manual and mechanical) caused hypoxia, which may have been distressing to birds because reflexes persisted for 43 ± 11 s in broilers killed by manual cervical dislocation and 106 ± 7 s in hens killed by mechanical cervical dislocation using a burdizzo.

Ideally, death would be confirmed by the absence of a heartbeat. Unfortunately, methods used to assess heartbeat, such as the ECG and Doppler flow detection, are not practical in field situations. Auscultation with a stethoscope may be used to monitor heartbeat, but the large pectoral muscles and broad sternum in birds dull the sounds from the heart (Heard, 2000), and this is made more problematic in turkeys due to genetic selection for very large breast muscles. During the course of the study, it was noted that sudden feather erection occurred in most turkeys during the tonic phase of convulsions. Because this was not part of the initial data collection, quantitative data on the time at which feather erection occurred was not consistently noted for all turkeys. This is unfortunate because it has been stated that sudden feather erection during anesthesia of birds is indicative of cardiac arrest or reduced blood flow to the heart (Heard, 2000). Gerritzen et al. (2007) used the occurrence of sudden feather erection, along with the absence of breathing and occurrence of tonic convulsions followed by complete muscle relaxation, to determine the time of death in poultry killed with CO_2. Although the significance of feather erection was not stated, future studies examining the occurrence of feather erection in combination with the ECG may be useful in determining whether sudden feather erection can be used as a practical indicator of cardiac arrest in the field.

In conclusion, the Zephyr appears to be a humane alternative on-farm killing method for cull turkeys. It is similar to blunt trauma in terms of the end time of convulsions and the proportion of large turkeys that regain sensibility but may be more consistent than blunt trauma at causing insensibility leading to death in small turkeys. The Zephyr also has the advantage of consistently administering the same force to the skull independently of the strength of the operator. These data provide the first quantitative assessment of the effectiveness of different killing methods for turkeys un-

der field conditions and can be used in the development of recommendations for humanely killing poultry.

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