Comparison of water-based foam and carbon dioxide gas emergency depopulation methods of turkeys

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ABSTRACT Recommended response strategies for outbreaks of avian influenza and other highly contagious poultry diseases include surveillance, quarantine, depopulation, disposal, and decontamination. The best methods of emergency mass depopulation should maximize human health and safety while minimizing disease spread and animal welfare concerns. The goal of this project was to evaluate the effectiveness of 2 mass depopulation methods on adult tom turkeys. The methods tested were carbon dioxide gassing and water-based foam. The time to unconsciousness, motion cessation, brain death, and altered terminal cardiac activity were recorded for each bird through the use of an electroencephalogram, accelerometer, and electrocardiogram. Critical times for physiological events were extracted from sensor data and compiled in a spreadsheet for statistical analysis. A statistically significant difference was observed in time to brain death, with water-based foam resulting in faster brain death ($\mu = 190$ s) than CO$_2$ gas ($\mu = 242$ s). Though not statistically significant, differences were found comparing the time to unconsciousness (foam: $\mu = 64$ s; CO$_2$ gas: $\mu = 90$ s), motion cessation (foam: $\mu = 182$ s; CO$_2$ gas: $\mu = 153$ s), and altered terminal cardiac activity (foam: $\mu = 208$ s; CO$_2$ gas $\mu = 242$ s) between foam and CO$_2$ depopulation treatments. The results of this study demonstrate that water-based foam can be used to effectively depopulate market size male turkeys.

Key words: depopulation, foam, electroencephalogram, turkey

INTRODUCTION

Highly pathogenic avian influenza and virulent Newcastle disease continue to cause poultry disease outbreaks. The major steps involved in controlling an outbreak such as highly pathogenic avian influenza include surveillance, quarantine, depopulation, disposal, and disinfection (USDA APHIS, 2012). Depopulation of infected birds or birds suspected of infection is used to eliminate animal suffering and stop virus replication and dissemination.

Turkeys are susceptible to avian influenza infections. Past avian influenza outbreaks in turkeys have resulted in significant economic losses. During the 1995 avian influenza outbreak in turkeys in Minnesota, 178 farms were infected, resulting in an economic loss of approximately $600,000 in 1 yr (Halvorson et al., 2003). Another low pathogenic avian influenza outbreak in 2002 affected 197 poultry farms in Virginia and cost an estimated $130 million in losses and cleanup. In that outbreak, turkeys accounted for 78% of the positive farms and bird losses (Virginia Department of Environmental Quality, 2002). Also in 2002, an H7N3 low pathogenic avian influenza outbreak on a turkey farm occurred in the Netherlands (Velkers et al., 2006). In 2007, an H5N1 highly pathogenic avian influenza outbreak on a large commercial meat turkey site stocked with 159,000 birds in Great Britain required depopulation (Irvine et al., 2007). From 2006 to 2008, there were 3 detections of avian influenza in turkeys in Canada (Senne, 2010). In 2007, water-based foam was used in Virginia and West Virginia to depopulate approximately 50,000 turkeys testing positive for H5N2 low pathogenic avian influenza in 2 separate incidents (Flory and Peer, 2010). In 2009, 2 turkey-breeding farms in Chile with an egg production decrease tested positive for H1N1 low pathogenic avian influenza. The Chilean outbreak was contained using strict biosafety measures and controlled marketing (Mathieu et al., 2010).

Methods of emergency mass depopulation need to maximize human health and safety while minimizing
the disease spread and animal welfare concerns. Selection of a method is dependent on the species, age, housing type, and disposal options available. No single method is suitable for all situations. The use of carbon dioxide gas for mass depopulation has been approved by the AVMA (2007). Gassing techniques using CO2, including containerized, whole house, and partial house, have been demonstrated for mass emergency depopulation (Kingston et al., 2005; Gerritzen et al., 2006; Raj et al., 2006; Sparks et al., 2010; Turner et al., 2012).

Water-based foam was conditionally approved for floor reared poultry by the USDA and American Veterinary Medical Association in 2006 (AVMA, 2006).

Water-based foam has been shown to be an effective method for depopulating chickens, ducks, chukars, and quail (Benson et al., 2007; Benson et al., 2009; Alphin et al., 2010; Benson et al., 2012a; Caputo et al., 2012). Flory and Peer (2010) documented that foam could be used in the field with market-weight turkeys; however, limited physiological data was available. The objective of this study was to evaluate the effectiveness of CO2 gas and water-based foam to depopulate market-weight turkeys.

MATERIALS AND METHODS

This study was conducted using a randomized block design with commercial male turkeys treated with 1 of 2 randomly assigned treatments: 100% CO2 gas or water-based foam with ambient air. The 48 turkeys used in this study were Hybrid Converter (Jansen Farms Hatchery Inc., Zeeland, MI) commercial males, 15 to 26 wk old, ranging in weight from 8 to 18 kg. Bird age was selected to represent market-weight and -age turkeys. Beginning at 15 wk of age, 4 turkeys were randomly selected per week for surgery and depopulation. Each turkey was instrumented with an accelerometer, electrocardiogram (ECG) electrodes, and a surgically implanted electroencephalogram (EEG) transmitter as described herein. Each turkey was placed individually in a 265-L (70 gal) chamber, a 60-s pretreatment baseline was recorded, 1 of 2 treatments (foam or CO2 gas) was applied, and sensor data was recorded for a total of 900 s (15 min). Treatment was performed within a chamber to allow treatment to be rapidly implemented while recording detailed physiological data. The time it took to reach motion cessation (accelerometer), unconsciousness (EEG), brain death (EEG), and altered terminal cardiac activity (ECG) was extracted from the sensor data. Treatment order was determined using a randomization table in Excel (Microsoft Corp., Redmond, WA). All testing was performed under the approval and guidelines of the University of Delaware Agricultural Animal Care and Use Committee and followed the guidelines laid out by the Federation of Animal Science Societies (2010).

The time to reach 4 critical physiological points was measured using the 3 sensors described previously. Motion cessation was determined from the accelerometer data, unconsciousness and brain death were determined using raw and frequency domain EEG data, and time to the onset of altered terminal cardiac activity (ATCA) was determined from ECG data. Altered terminal cardiac activity was the initial point of disruption to the ECG signal, from which recovery was not possible.

Data Analysis

For data analysis, critical times for physiological events were extracted from the EEG, ECG, and accelerometer data as described above, compiled in Excel, and statistical analysis was performed using SAS (SAS Institute Inc., Cary, NC). The SAS data set was coded to extract sensor data valid for treatment analysis. The extracted treatment data was used to determine the distribution of analysis-specific data sets. The data subsets were not normally distributed and, thus, a nonparametric statistical analysis was conducted on each data subset in SAS. A Wilcoxon exact test and Student’s t-test was used to analyze the treatment-dependent data sets. All tests were conducted at the 5% (α = 0.05) significance level.

General Procedure and Instrumentation

Approximately 24 to 48 h before a trial, 4 turkeys were randomly selected to have food withheld for approximately 8 h and water withheld for approximately 2 to 6 h before surgery. Each turkey was anesthetized using 5% isoflurane (IsoSol; Vedco Inc., St. Joseph, MO) at induction with 3% isoflurane for maintenance of anesthesia. Three-channel wireless biopotential transmitters (PhysioTel model F50-EEE, Data Sciences International, St. Paul, MN) were surgically implanted in the back of the neck of each turkey. Three leads were placed on the meninges covering the telencephalon through 0.9-mm holes that were drilled into the parietal bone, 2 holes on the right side of the midline and one on the left, using a high speed microdrill (model 18000 17, Fine Science Tools, Foster City, CA). Two leads were implanted in the complexus muscle just below the base of the skull for electromyography (EMG). The turkeys were given an injection of 0.4 mg/kg of carprofen subcutaneously after the procedure and were allowed to recover for 24 h. The surgical procedure was based on Savory and Kostal (1997, 2006) and Alphin et al. (2010).

Signals from the wireless transmitter were recorded by 2 RMC-1 PhysioTel (Data Sciences International) receivers placed opposite one another at the bottom of a 265-L (70 gal) chamber and 2 additional receivers placed opposite one another at approximately 0.9 m (3 ft.), the approximate height of a market-weight turkey head. The signals from the receivers were passed through a Matrix (Data Sciences International). Brain activity was monitored and recorded using Dataquest A.R.T. Acquisition software (Data Sciences International). Brain activity files were processed and analyzed.
in NeuroScore (Data Sciences International) to detect EEG silence, brain death, and unconsciousness.

The raw EEG and EMG signals were analyzed in NeuroScore by adding labeled markers over artifact-free 2-s epochs indicating pretreatment (first 60 s), posttreatment, convulsion, and postconvulsion periods. The markers were placed based on visual analysis of the EEG signal using the EMG signal as a reference to eliminate motion artifacts, which appear as high amplitude spikes in both the EEG and EMG channels. The mean EEG, mean EMG, α (8–12 Hz), β (16–24 Hz), δ (0.5–4 Hz), θ (4–8 Hz), and σ (12–16 Hz) values and markers were exported on a 2-s basis from Neuroscore to Excel and charted. Electroencephalogram silence was determined to be the point at which the mean signal over 1-s periods was stable (minimal to no change) at about 0 mV, which is an indication of brain death. Time to unconsciousness was extracted from the frequency domain EEG data file utilizing the relative power band ratio α-to-δ, which monitors a trend from high- to low-frequency brain activity (Benson et al., 2012b,c).

An accelerometer was attached to the left leg to measure motion cessation. Two different shear mode accelerometers were used interchangeably. The model 353B16 (PCB Piezotronics, Depew, NY) accelerometer had a sensitivity of 1.02 mV·s²/m ± 10% (10 mV/g ± 10%) capable of operating over a range of ±4,905 m/s² (500 g) of peak. The higher sensitivity model 352C66 (PCB Piezotronics) accelerometer operated at 10.2 mV·s²/m ± 10% (100 mV/g ± 10%) over a range of ±491 m/s² (±50 g) of peak. For depopulation, the differing sensitivities and operational ranges are inconsequential, as the signal characteristic of interest is a mean 0 V signal (flat line) occurring after convulsions. The output from the accelerometer was passed through a single-channel signal conditioner (model 480C02, PCB Piezotronics) connected to a data acquisition card (model PCI-6036E, National Instruments, Austin, TX). The conditioned signal was collected at 100 Hz in a custom written LabVIEW (National Instruments) virtual instrument. Text files generated by the virtual instrument were processed through a custom program written in Visual Basic for Applications in Excel (Microsoft Corp.) to reduce the signal frequency and chart the data. The motion cessation procedure was based on Dawson et al. (2007, 2009).

To measure electrical cardiac activity, each turkey was instrumented with ECG electrodes and leads (Biopac Systems Inc., Goleta, CA) placed on a previously plucked area on each leg and underneath the right wing. ECG signals were processed through an MP30A acquisition unit (Biopac Systems Inc.) and recorded using Biopac Student Lab software. Analysis of the ECG signals was conducted using Biopac Student Lab Pro to analyze the recorded signals and to find the onset of ATCA. Altered terminal cardiac activity was the cessation of rhythmic electrical cardiac activity that invariably results in an isoelectric ECG. The point of ATCA was selected to be after terminal convulsions and associated motion artifact cease, and arrhythmic electric activity begins (Caputo et al., 2012).

**Foam and CO₂ Gas Treatment Specifics**

For the foam treatment, water-based foam with ambient air was created using a nozzle type foam depopulation system (model AG-1, Spumifer, Ridgefield Park, NJ). This system draws air through the rear of the nozzle and combines it with a mixture of the foam concentrate and water. A 1% solution of foam concentrate (WD-881, Phos-Check, St. Louis, MO) and water was premixed on the day of trial. A Darley water pump (model 2 1/2 AGE 31 BS, Darley, Itasca, IL) was used to supply the required pressure and flow. The pump was driven by a 23-kW (31 hp) Briggs & Stratton (Milwaukee, WI) Vanguard gasoline engine providing a rated performance of 1,136 L/min (300 gal/min) at 586 kPa (85 psi). This foam system meets the USDA-Animal and Plant Health Inspection Service conditional requirements for water-based foam depopulation. Foam was applied until the 265-L (70 gal) chamber was full. For the CO₂ gas treatment, the top of the chamber was covered with a 0.64-cm (1/4 in.) sheet of transparent polycarbonate, allowing observation of the birds during gas depopulation. The gas was introduced at 2,265 L/min (80 ft³/hr) until the turkeys displayed terminal convulsions and motion cessation.

**RESULTS**

Figure 1 summarizes the time from treatment to each of the 4 the physiological parameters evaluated in this study. Several data sets were eliminated from analysis due to signal noise, irregularities, or sensor errors. Water-based foam was faster than CO₂ for unconsciousness, brain death, and ATAC; however, only the time to brain death with foam (μ = 190 s) was statistically significantly faster than CO₂ gas (μ = 242 s; P < 0.0025). Water-based foam resulted in faster (μ = 64 s) time to unconsciousness than CO₂ gas ( μ = 90 s), but the difference was not statistically significant (P < 0.2286). Water-based foam was also faster (μ = 200 s) to ATCA than CO₂ gas (μ = 220 s), but again the results were not significant (P < 0.1559). Carbon dioxide gas was faster to motion cessation (μ = 153 s) than water-based foam (μ = 182 s), although the results were not significant (P < 0.2236). Bird age was not a factor for ATCA (P > 0.2844), EEG silence (P < 0.7983), or unconsciousness (P < 0.6639), and was a limited factor for motion cessation (P < 0.0941).

**DISCUSSION**

Water-based foam was shown to be effective for depopulating turkeys. Whole house, partial house, and containerized CO₂ gassing are currently considered acceptable depopulation methods for floor-reared birds,
FOAM DEPOPULATION OF TURKEYS

Figure 1. Mean time, in seconds, to the physiological points unconsciousness, motion cessation, brain death, and altered terminal cardiac activity (ATCA) for adult tom turkeys depopulated with either foam or CO₂ gas. Data are presented as mean ± SEM. Letters (a–e) denote statistical significance between treatments within physiologic variables ($P < 0.05$).

including turkeys. In the metrics evaluated, water-based foam required the same amount of time or was faster than CO₂ gas for most metrics. Time to unconsciousness is indicative of how long birds are aware of their surroundings and the depopulation process. Processes that result in faster times to unconsciousness are considered more humane when all other factors are equivalent. As water-based foam and CO₂ gas require approximately the same amount of time to unconsciousness, both processes leave the bird aware for similar lengths of time.

The time to motion cessation for carbon dioxide gassing and water-based foam was not statistically different. Motion cessation is important because it (1) can be reliably implemented during depopulation, (2) requires little interpretation to evaluate the results, and (3) can be mapped to other physiological markers. Brain death was the 1 metric out of 4 where a statistically significant difference was observed between treatments, with foam being faster than CO₂ gas. Faster time to brain death indicates reduced exposure during depopulation, with faster processes being more humane than slower processes. Continued cardiac activity can occur after brain death in poultry. Altered terminal cardiac activity or cardiac suppression occurs after brain death, but before cardiac arrest. This is another metric to compare depopulation procedures; however, this event is not as significant as the 3 previous metrics. The time to ATCA was not statistically different for foam and CO₂ gas.

Differences exist between individual depopulation and mass emergency depopulation. Individual depopulation allows better scientific control and improved evaluation of individual birds. Mass emergency depopulation allows assessment under real world conditions. In extending results from individual to mass emergency depopulation, foam and CO₂ gassing scale differently.

For foam depopulation, treatment typically starts at one end of the house and foam is applied to create a cover over and around the birds. Once an individual bird is treated, the bird is affected and times to unconsciousness, motion cessation, brain death, and ATCA for the individual bird are similar to the results for this study. The treatment process continues and additional birds are treated with foam until all birds are covered. Time for treatment of the entire flock or building is based on time to achieve head coverage of the birds. Individual birds; however, are only exposed for a short time after treatment before unconsciousness and brain death. In contrast, for gassing procedures, the gas concentration has to increase to a level necessary to cause unconsciousness before treatment begins to affect the birds. Gas concentration can vary spatially across the treatment zone, and gas concentration, application rate, and treatment zone sizes determine the speed at which concentration increases. For this reason, individual and mass emergency depopulation times for gassing procedures do not scale well. Whereas water-based foam depopulation and CO₂ gassing of adult turkeys require similar times for individual birds, the time to key physiological events may be longer during mass emergency depopulation for CO₂ gassing than water-based foam (Benson et al., 2007).

During the course of a low pathogenic avian influenza response in West Virginia in 2007, Flory and Peer (2010) discussed problems that occurred when nozzle-applied water-based foam was used to depopulate market-weight turkeys of approximately 18 kg. Flory and Peer (2010) documented the effectiveness of the large foam generators in depopulating market-weight turkeys. The difference in effectiveness of the 2 systems was attributed to the lack of experience of the emergency response contractors with the foam-generating equipment. This study demonstrates that nozzle-applied water-based foam can be used to depopulate market-weight turkeys. This, combined with results from Flory and Peer (2010) for large foam generators, demonstrates that properly applied water-based foam is able to effectively depopulate market-weight turkeys.

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