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Application of In-House Mortality Composting on Viral Inactivity of Newcastle Disease Virus

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ABSTRACT This paper summarizes the results from 3 simulated in-house catastrophic mortality composting experiments. Experiment 1 evaluated the impact of water-based foam mass depopulation on in-house composting of the carcasses and litter and showed that water-based foam improved windrow temperatures. Experiment 2 evaluated the impact of freezing samples on virus recovery from windrow compost tissue and the choice of tissue for virus sampling within the bird. Experiment 2 documented that freezing the samples had minimal impact on processing and that virus recovery was more consistent among inoculated breast meat than inoculated tracheas. Experiment 3 evaluated the impact of sawdust, straw, and sawdust-straw base layer litter material on in-house mortality composting. All litter materials were able to reach and maintain temperatures in excess of 60°C for multiple days. No viral hemagglutination activity was observed after d 2 during any of the 3 experiments.

Key words: compost, windrow, in-house, inactivation, Newcastle disease virus

INTRODUCTION

The possibility of an avian disease outbreak is always a concern for the poultry industry. Avian influenza virus (AIV) and velogenic Newcastle (also known as exotic Newcastle disease virus, END) are significant threats to the US poultry industry. In particular, H5N1 AIV has been confirmed in Asia, Europe, and Africa and continues to spread to other countries. Between 2003 and 2007, there were over 340 confirmed avian influenza H5N1 cases and 208 human deaths across the world (WHO, 2007). In 2004, over 100 million poultry were culled worldwide due to highly pathogenic AIV (HPAIV) or low-pathogenic AIV (LPAIV; Clark and Hall, 2006). Between 2002 and 2003, an outbreak of END in California resulted in the quarantine of 18,345 premises, the culling of over 3.2 million birds, and cost over $170 million (Breitmeyer et al., 2003).

Control of AIV, END, and other highly contagious poultry diseases consists of detection, confinement, and depopulation or vaccination of the affected region. Quarantine of the area is an immediate step in control of an infectious poultry disease and can significantly reduce the spread of the disease. Birds that are infected or suspected of infection are depopulated using the most expedient method possible. Vaccination has been used in control of several outbreaks; however, the usefulness of conventional vaccination as an eradication tool for avian influenza is unclear (Capua and Alexander, 2004).

Mass emergency depopulation is often required as part of a response to highly contagious poultry diseases. Current procedures for large-scale emergency depopulation of meat-type birds consist of exposing poultry to CO2 gas. These procedures include, but are not limited to, the following: a) whole- or partial-house gassing, b) containerized gassing techniques, and c) the polyethylene tent method. Whole- and partial-house gassing requires sealing the house to prevent gas leakage, the use of specialized equipment, and the quick introduction of large volumes of gas evenly over the birds (Kingston et al., 2005). Containerized procedures typically require hand-catching of the birds, placement of the bird into containers, and applying gas to the sealed containers. The polyethylene tent method, which has been used for broilers, utilizes overlapping layers of polyethylene sheeting to cover birds, forming a tent over the birds, and filling the environment under the sheet with CO2. An alternative depopulation procedure using water-based foam was developed to improve biosecurity, reduce labor requirements, and decrease the number of people exposed to potential zoonotic agents. Compared with gassing procedures, the water-based foam procedure is generally faster, shows similar bird stress, is more biosecure, and requires fewer people (Dawson et al., 2006; Benson et al., 2007).
After depopulation, disposal of the carcasses and decontamination of the facilities are required. Disposal options for mass emergency carcass disposal include pit burial, landfilling, incineration, rendering, and composting (Sander et al., 2002). Biosecurity, transportation logistics, public perception, and environmental concerns limit the choice of disposal methods. In addition, movement of infected carcasses is believed to have been responsible for the spread of LPAIV during the 2002 Virginia outbreak (Senne et al., 2003). Composting is a sanitary and practical method of carcass disposal that can inactivate many pathogens and result in a soil amendment.

Composting is defined as the natural decomposition of organic materials by aerobic bacteria and fungi (DeRouchey et al., 2005). Composting for animal mortality includes the temporary burial of dead animals above ground in a mound of supplemental C and allowing decomposition by thermophilic microorganisms to heat up the pile, kill most pathogens, and digest the carcass tissues under predominantly aerobic conditions (Kalbasi et al., 2005). The composting process generally follows 2 phases: a heating (or developing) phase and a curing (or maturation) phase. In the heating phase, there is high O₂ consumption, thermophilic temperatures (>55°C), and a rapid reduction in biodegradable solids. The maturation phase, during which aeration is less of a factor and slower reactions occur, is typically not conducted inside the house during in-house composting. For optimal composting, a C:N ratio of between 25:1 to 30:1, moisture content within the range of 50 to 60% (wt/wt), porosity of 35 to 45%, and O₂ levels of 10% volume are recommended (Wilkinson, 2007).

Most composting windrows are periodically turned or rotated to increase aeration and expose the anaerobic zone in the core to air. Thermophilic conditions are reached during mortality composting, but these windrows are generally left undisturbed during the first or primary stage of composting as soft tissue decomposes. Turning increases the amount of material exposed to the core of the windrow and increases organic material degradation rates. The second composting stage is used to break down the remaining bones and materials (Wilkinson, 2007). The timing of the turning of the composting windrow is typically based on temperature monitoring of the composting windrow (Malone, 2006).

Composting has been demonstrated as an effective mass emergency disposal option for animal carcasses and, in particular, for poultry. Murphy (1992) reported success with in-house composting of 86,000 two-kilogram broilers on a 4-house commercial farm. After 10 d in the windrows,
the compost material from this agricultural herbicide-contaminated flock was removed, applied to cropland, and incorporated as fertilizer. During the 2002 H7N2 LPAIV outbreak in the central Shenandoah Valley, approximately 43,000 birds from 2 flocks were composted using both in-vessel (Ag-Bag) and in-house composting. During the 2004 H7N3 HPAIV outbreak in British Columbia, approximately 46% of the infected carcasses were disposed of using on-farm composting (Wilkinson, 2007). In-house composting was used in 2004 as part of a response during a LPAIV H7N2 outbreak on Delmarva to successfully contain and inactivate the virus in the carcasses and litter (Malone et al., 2004; Malone, 2006). Although composting has significant advantages for catastrophic mortality disposal associated with a disease outbreak, composting can be used to dispose of catastrophic mortality due to heat stress, floods, and chemical residues (Malone, 2006). In-house composting has been used to dispose of 5 flocks lost to catastrophic heat stress in Virginia since 2002 (Bendfeldt et al., 2006).
Composting is an established pathogen reduction technology. For catastrophic carcass composting, the Canadian Food Inspection Agency has suggested using the term “bio-heat treatment” to clarify that the composting process produces heat, NH₃, and other products that can kill viruses (Spencer et al., 2004). Composting has been shown to control nearly all pathogenic viruses, nonendospore-forming bacteria, fungi, and protozoa (Wilkinson, 2007). Time and temperature are required to achieve viral inactivation. Typical contact times within compost windrows are measured in days, and studies have shown that HPAIV [A/CK/PA/1370/83 (H5N2)] was inactivated after 10 d of carcass composting (Senne et al., 1994). In the Senne experiment, the average temperatures recorded during the composting process were 57.3°C on d 4 and 58.3°C on d 13 in the upper layer of birds and 41.5°C on d 6 and 42.8°C on d 17 in the lower layer of carcasses. No virus was isolated from any organ material, indicating inactivation occurred. Newcastle disease virus is also heat-labile, and the inactivation time is dependent on virus strain, surface or medium, and virus titer and inversely related to temperature (Swayne and Beck, 2004). They are enveloped viruses with glycoprotein projections from the envelope that have hemagglutinating and neuraminidase activity. Being enveloped viruses, both NDV and AIV are inactivated by heat, oxidation, and pH effects (Alexander, 1997; Swayne and Halvorson, 1997). Because of their similar architecture and environmental stability, NDV is used as a surrogate virus for AIV in field studies. In addition, the threat of END makes the use of NDV for field trials appropriate.

A variety of supplemental C materials have been successfully used to compost animal carcasses including hardwood sawdust, woodchips, built-up litter, straw, corn stalks, and peanut hulls (Kalbasi et al., 2005; Bendfeldt et al., 2006). Hardwood sawdust- and wood shavings-based poultry litter are typically recommended as an ideal C source for composting (Wilkinson, 2007). Tablante and Malone (2005) found that with the mix-and-pile method, a minimum of 2.1 cm of base litter material was required for each 5 kg of meat per square meter of floor space for in-house composting. With larger birds (i.e., roasters or turkeys) or with the layering method of composting, the factor increased to 2.6 cm of base litter for each 5 kg of meat per square meter of floor space.
Straw has been used as an additive for compost windrows; however, straw does not always reach the required temperatures for inactivation. In a study performed with adult dairy cows, the effectiveness of straw and sawdust as supplemental C sources was compared (Granatstein, 1999). Straw bedding resulted in faster and more complete decomposition of the carcass but resulted in lower pile temperatures than sawdust. Less water was used during the construction of the straw pile (52 L) than the sawdust pile (227 to 264 L). The peak temperatures were approximately 60°C for sawdust and 49°C for straw. Catastrophic cattle mortality windrows created with corn silage rapidly reached inactivation temperatures and maintained temperatures between 60 and 70°C for protracted periods of time (Glanville et al., 2006b). In contrast, composting windrows made with ground cornstalks or straw and manure were generally 10° to 20°C cooler and took longer to reach inactivation temperature when moisture levels were lower.

This paper presents the results from 3 experiments in which in-house composting was used to inactivate NDV in simulated catastrophic mortality events. Experiment 1 evaluated the impact of water-based foam mass depopulation and in-house composting. Experiment 2 evaluated the impact of freezing samples on virus recovery and the choice of tissue within the bird. Experiment 3 evaluated the impact of using 1 flock litter from sawdust and straw base on mortality composting.

**MATERIALS AND METHODS**

**Experiment 1**

Experiment 1 was conducted as part of an industry-sponsored water-based foam depopulation demonstration. The purpose of the demonstration was to evaluate large-scale water-based foam depopulation and to determine if the added water or foam concentrate from the foam depopulation procedure interfered with carcass or mortality composting. Two sample foam depopulation systems were evaluated under commercial conditions for their ability to depopulate meat-type chickens. Each system was used to depopulate an equivalent number of birds (2,600, 2.7-kg broilers) in the same size area (12.5 × 19.2 m, 240 m²). Both treatments were conducted on used peanut hull base litter, and pine sawdust was used to cap the windrows. An equal number of approximately the same size processing plant dead-on-arrival carcasses served as a non-treated control. During the depopulation demonstration, the birds were treated with a 1% mixture of water-based fire-fighting foam concentrate. The mix-and-pile composting procedure was used to form the windrows using the equivalent of 2.1 cm of base litter material for each 5 kg of meat per square meter.

A continuous compost pile including 3 distinct separate treatments was created using the broilers and litter from
the depopulation demonstration. Three treatments were used (T1—foam generator with Chemguard concentrate, T2—nozzle system with Phos-Chek concentrate, and T3—control, no foam). Treatment 1 (T1) used an Avi-Foamguard (Kifco Company, Havana, IL) foam generator depopulation system utilizing Chemguard (Mansfield, TX) foam concentrate injected at a 1% rate. For T1, approximately 3,458 L of water and 34 L of foam were required to fill the space, or about 14.4 L/m² of water and 0.14 L/m² of foam concentrate. Treatment 2 (T2) used a Spumifer AG-1 style aspirated nozzle system (Ridgefield Park, NJ) and Phos-Chek (St. Louis, MO) foam concentrate mixed at 1% concentration. For T2, approximately 3,702 L of water and 38 L of foam were required, or about 15.4 L/m² of water and 0.16 L/m² of foam concentrate. The birds from the depopulation experiment were used to create a continuous compost windrow. For sampling purposes, a 3-m buffer zone was allowed to separate each treatment. The control treatment (T3) was made up of a roughly equivalent number of poultry processing plant dead-on-arrival birds.

Temperature data were collected using Global Sensors Model DW-2E-D-16 (Global Sensors LLC, Belmont, NC) with sensors at duplicate locations at depths of 2.5, 30, and 90 cm.

**Experiment 2**

Experiment 2 was conducted to evaluate the impact of freezing the samples on virus isolation and to evaluate whether whole birds were required for a valid sample. During actual outbreak situations, it is often not practical to analyze large numbers of fresh samples, and samples may need to be frozen before analysis. In addition, although inclusion of tracheas may be appropriate for evaluation of upper respiratory viruses, breast meat can be easily analyzed using the same equipment listed in experiment 1. An estimate of carcass degradation and odor was performed at 2 and 4 wk.

**Experiment 3**

Experiment 3 explored the use of litter from alternative bedding materials and the impact on in-house carcass or mortality composting. In particular, the experiment explored the use of a straw base bedding material that is common for many poultry production areas of the world. An estimated 3,600 kg of processing plant dead-on-arrival birds was used to simulate catastrophic mortality. This represented approximately one thousand four hundred 2.6-kg birds. Using an estimated rearing density of 32.8 kg/m² and a litter base factor of 2.1 cm per 5 kg/m², an equivalent of 13.8 cm of litter base was used for this catastrophic composting experiment. A windrow measuring 22.9 × 4.3 × 15.2 m (height × width × length) with a 15-cm-deep base layer was constructed inside a covered manure shed. Three treatments (T1—sawdust base litter, T2—50% sawdust:50% straw litter, and T3—straw litter) were used. Each treatment was approximately 3 m long with 1.5 m of litter; there was no mortality buffer between treatments.

To simulate a 1-flock litter base using a 7.6-cm sawdust or straw bedding base, compost windrow material was premixed 2 wk before set up of the mortality composting
experiment. Treatment T1 included 3.8 m³ of sawdust, 1.3 m³ of manure, and 1.7 m³ of mortality. Treatment T2 included 1.9 m³ of sawdust, 3.8 m³ of straw, 1.3 m³ of manure, and 1.7 m³ of mortality. Treatment T3 included 7.7 m³ of straw, 1.3 m³ of manure, and 1.7 m³ of mortality. Manure came from a 14-flock litter base. The mortality addition was held constant for each treatment and was performed 2 wk after mixing the base materials. Treatments T1 and T2 were capped with sawdust, whereas T3 was capped with straw after construction. The windrows were turned at 2 and 4 wk (final assessment and termination of the experiment) and recapped with sawdust or straw based on treatment.

Compost temperatures were monitored at 3 depths (2.5, 30, and 90 cm) and 2 locations per treatment using the equipment and procedure described in experiment 1. An estimate of carcass degradation and odor was performed at 2 and 4 wk.

Approximately 30 broiler breasts were collected from a previous vaccine challenge study. Each breast was inoculated with 0.2 mL of 10⁹ EID₅₀ of low passage field strain of LaSota NDV at 2 sites per breast. The injection sites were marked with plastic wingbands to allow tissue samples to be extracted from the injection site. One inoculated breast and 2 wet virus sample tubes were wrapped in a hair net and then placed in plastic mesh produce bags, which served as sample bags. Sample bags were placed in each treatment at 2 placement locations (near the bottom and near the top) of the compost pile. The sampling day and sampling date were placed on ID cards located both inside the bag and on a nylon cord tied around the sample bag and visible from the outside of the windrow. Samples were placed approximately 0.3 m inside the pile and covered with litter material. Samples were collected from each treatment and placement location on d 0, 1, 2, 3, and 4. Wet positive control sample tubes containing 2.0 mL of dilute NDV were placed inside the building but outside of the windrow. Two sample tubes were collected on d 0, 1, 2, 3, and 4. The compost and wet positive control sample tubes were frozen immediately after sampling. The remainder of the virus reisolation procedure was the same as for experiment 2.

RESULTS AND DISCUSSION

The compost piles used in all 3 experiments were able to successfully dispose of catastrophic mortality. Virus recovery from the experimental treatments decreased with time, and no hemagglutinating-positive samples were recovered after d 2 in any treatment. The rapid reduction in NDV was faster than other large animals, outdoor windrow studies have shown (Glanville et al., 2006b).

Experiment 1

Experiment 1 documented that fire-fighting foam did not adversely impact the use of in-house composting of birds. In fact, the added water from the foam solutions (T1 Chemguard and T2 Phos-Chek) may have contributed to higher temperatures than the no-foam control treatment (Figure 1). The compost windrow went through a rapid temperature rise after creation, with an initial heating period of 2 d. The compost windrow was turned at d 14, resulting in a spike in windrow temperatures for all treatments.

Prior research has shown that AIV and NDV can be inactivated by heat. Avian influenza virus treated by heat was not detected after 30 min at 60°C and could not be detected after 60 min at 56°C. Newcastle disease virus shows similar thermal inactivity to AIV and can be thermally inactivated after 30 min at 60°C under laboratory conditions; however, in samples with high virus concentration, NDV strains were not inactivated by treatment at 56°C for 120 min (King, 1991). Additional research has shown that HPAIV virus titers remained unchanged in breast and thigh meat at temperatures of 30, 40, and 50°C, although reductions in titer were identified at 60°C (Swayne, 2006). At 70°C, less than 5 s was required for inactivation. At shallow treatments (2.5 and 30 cm), the foam treatments met or exceeded recommended windrow treatments for virus inactivation (55°C). At shallow temperatures, the control treatment did not meet the required temperatures. At deeper depths (90 cm) in the windrow, all 3 treatments met or exceeded the recommended temperature and maintained the recommended treatment. Turning of windrow helped to increase maximum windrow temperatures. For all treatments, approximately 95% of the carcass tissue was degraded by the first 2 wk of composting, with no offensive odor observed for any compost material.

The significance of the temperature differences was analyzed. The temperatures, depths, and treatments were statistically different (Microsoft Excel ANOVA, α = 0.05). At the shallowest depth (2.5 cm), the foam treatments were not statistically different (Microsoft Excel t-test, α = 0.05). At all other depths, the temperature of the foam treatments was statistically different and higher than the control group. At all depths, the foam treatments were statistically different than the control treatment both individually and when grouped together. Because mass mortality composting using litter as the bulking agent is often deficient in moisture, this indicates that the addition of water from the foam solution helped raise compost windrow temperatures. In addition, this showed that foam concentrate did not have an adverse impact on composting. The higher temperatures should help to promote virus inactivation.

Experiment 2

During experiment 2, a single treatment was used. Experiment 2 indicated that trachea and breast samples behaved comparably as sampling locations. In addition, experiment 2 indicated that samples could be analyzed while fresh or after freezing.

The temperature of the composting windrow varied slightly between locations (Figure 2). The temperatures within the pile exceeded 50°C in all portions of the composting windrow except shallow south. Peak temperatures
for both shallow north and deep north reached and maintained temperatures exceeding 55°C for 4 or more days. Peak temperatures in shallow south reached 49°C but maintained the temperature for 3 d. The compost windrow constructed for experiment 2 was relatively small and did not have the thermal mass of the larger windrows constructed for experiments 1 and 3. The shallow south temperature reading was most likely not located deep enough within the pile and was adversely impacted by ambient temperature.

The samples showed positive hemagglutination activity through d 2 (Figure 3). There were fewer positive samples in the fresh samples than the frozen samples. The high contaminant load in the compost windrow lead to bacterial contamination of the samples, and the bacterial contamination resulted in egg mortality. Egg mortality immediately after inoculation cannot be separated from bacterial contamination, live virus, or shock due to inoculation. As a result, the high mortality was not detected after samples were being collected for the additional days. To compensate for the high containment load in the compost windrow, the antibiotic contact time and centrifuge rates were increased and the virus reisolation repeated. The changes in procedure were conducted while the fresh samples were being analyzed. The frozen samples were completed after the procedure had been modified and were not significantly impacted by contamination. The initial concern was that freezing the samples would tend to decrease virus survivability due to rupturing of the lipid envelope. The frozen samples showed more hemagglutinating activity under otherwise similar conditions. This indicates that freezing does not adversely affect hemagglutinating activity.

Trachea and breast meat samples were placed in the composting windrow for sampling. Frozen samples were the most consistent. Trachea and breast samples showed similar hemagglutinating activity in frozen samples. When positive hemagglutinating activity was recovered from one location, positive hemagglutinating activity was recovered from the other location.

This study showed that freezing the samples and performing reisolation at the conclusion of the study would not have an adverse effect on virus hemagglutinating activity. This is important, because during an outbreak response, immediate analysis of fresh samples cannot always be achieved. In addition, the study showed that inoculated breast meat could be used in place of tracheas for field composting studies. Inoculated breast meat is easier to secure and prepare for field studies, in particular, studies connected with industry mortality events.

**Experiment 3**

Experiment 3 showed that sawdust, sawdust-straw mixtures, and straw base 1-flock old litter could be satisfactorily used to compost catastrophic mortality.

The temperature in all 3 treatments exceeded 60°C for multiple days (Figure 4). The temperature in all 3 treatments increased after turning of the windrow. The temperatures for all treatments did not go below 50°C for any treatment until over 20 d had passed. The temperature in the straw showed the highest peak temperatures, the most variability, and a difference in profile versus other materials. Straw peaked at over 70°C, which is considered very high for a composting experiment. Granatstein et al. (2006b) suggest that temperatures in excess of 60°C may cause the microorganisms to die or go dormant, reducing the overall decay rate. The temperature profile for straw does not match average ambient temperatures for the testing location (results not shown). The results from experiment 3 disagree with the results from Granatstein (1999), in which windrow temperatures created with straw material did not exceed 50°C during the course of the experiment. Experiment 3, however, used straw base litter, and Granatstein used wheat straw as the bulking agent. In experiment 3, the windrow temperatures consistently exceeded the minimum-suggested virus isolation temperature of 55°C. Tissue degradation for all treatments at the 2-wk turning was estimated at 97%, with odor being characterized as a sweet, nonoffensive aroma. By 4 wk, all soft tissue was degraded for all treatments.

Virus isolation showed that little hemagglutinating activity was detected in the compost pile (Figure 5). Virus reisolation showed hemagglutinating activity from the sample on d 0; however, minimal hemagglutinating activity was detected in the windrow after d 0. One positive sample was recovered from straw on d 1. No positive samples were recovered after d 1, largely due to the high temperatures achieved within the windrow.

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