





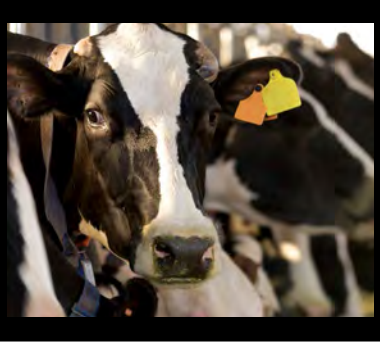
6th International Sympoisum on Animal Mortality Management

A triennial symposium since 2005

June 3-7, 2018

Embassy Suites Hotel
Amarillo, Texas

animalmortmgmt.org

Thank you to our dedicated committee members for making the 2018 Symposium a success!

Steering Committee:

- Gary Flory, Co-Chair, Virginia Department of Environmental Quality
- Mark King, Co-Chair, Maine Department of Environmental Quality
- Robert DeOtte, West Texas A&M University
- Jean Bonhotal, Cornell Waste Management Institute
- Mark Hutchinson, University of Maine Extension
- Edward Malek, Canadian Food Inspection Agency
- Lori Miller, United States Department of Agriculture, Animal and Plant Health Inspection Ser vice, Veterinary Services Science, Technology and Analysis Services
- Joshua Payne, Jones-Hamilton Ag.
- Dale Rozeboom, Michigan State University
- Megghan Honke Seidel, Michigan State University
- Mike Mayes, North Carolina Department of Agriculture & Consumer Services

International Program Sub-Committee:

- Gary Flory, Chair, Virginia Department of Environmental Quality
- Dr. Ndubuisi Machebe, University of Nigeria, Nigeria
- Dr. Mohamed Naceur Baccar, National Center of Zoosanitary Vigilance, Tunisia
- Mr. Duncan Worsfold, Department of Environment and Primary Industries, Australia
- Dr. Eran Raizman, Food and Agriculture Organization of the United Nations, Italy
- Mr. Edward Malek, Canadian Food Inspection Agency
- Dr. Van Dang Ky, Past Chief of Epidemiology, Vietnam

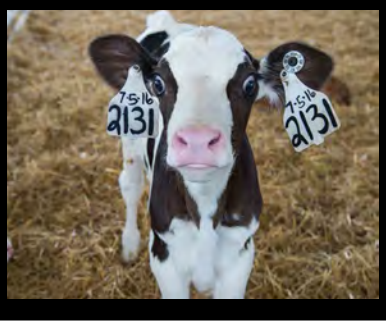
Venue/Tours Sub-Committee

- Robert DeOtte, Co-Chair, West Texas A&M University
- Joshua Payne, Co-Chair, Jones-Hamilton Ag.
- Ben Weinheimer, Texas Cattle Feeders Association
- Darren Turley, Texas Association of Dairymen
- Brandon Gunn, Texas Pork Producers Association
- Ty Lawrence, West Texas A&M University

Vendor and Exhibitor Sub-Committee

- Mark Hutchinson, Chair, University of Maine Extension





Exercise Sub-Committee

- Edward Malek, Chair, Canadian Food Inspection Agency
- Robert DeOtte, Co-Chair, West Texas A&M University
- Kathryn Willcutts, Planner/Facilitator, U.S. Department of Homeland Security
- Ben Weinheimer, Texas Cattle Feeders Association
- Sandy Johnson, Kansas Department of Agricluture
- David Solis, Texas Division of Emergency Management
- Walt Kelley, Retired Emergency Manager – Amarillo
- John Kiehl, Panhandle Regional Planning Commission
- Brian LeLande, USDA APHIS Veterinary Services
- Buck Hamilton, U.S. Department of Homeland Security
- Gayman Helman, Texas A&M Veterinary Medical Diagnostic Laboratory

Program/Evaluation Sub-Committee

- Jean Bonhotal, Chair, Cornell Waste Management Institute
- Mary Schwarz, Cornell Waste Management Institute
- Robert DeOtte, West Texas A&M University
- Josh Payne, Jones-Hamilton
- Ken Powell, Kansas Department of Health and Environment
- Lori Miller, USDA APHIS Veterinary Services
- Rebecca Podgorski, Wisconsin Department of Environmental Quality
- Dale Rozeboom, Michigan State University
- Gary Flory, Virginia Department of Environmental Quality
- Mike Mayes, North Carolina Department of Agriculture and Consumer Affairs
- Zoe McManama, Wisconsin Department of Environment Quality

Demonstration Sub-Committee

- Mark King, Co-Chair, Maine Department of Environmental Quality
- Robert DeOtte, Co-Chair, West Texas A&M University
- Lori Miller, United States Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services Science, Technology and Analysis Services
- Bob Peer, Virginia Department of Environmental Quality
- Mike Mayes, North Carolina Department of Agriculture & Consumer Services
- Rebecca Podgorski, Wisconsin Department of Agriculture
- Zoe McManama, Wisconsin Department of Agriculture
- Brent Auvermann, Texas A&M AgriLife Research and Extension
- Mark Hutchinson, University of Maine
- Joe Hudyncia, North Carolina Department of Agriculture and Consumer Services




Agenda

Sunday, June 3, 2018

Reception and Registration

Monday, June 4, 2018

Breakfast and Registration/Load Buses

Texas Agriculture Tours

- A look at large-scale beef and dairy production and processin
- Explore Texas sized beef and dairy production with a peek into cheese processing
- Swine production and processing with a side of beef production

5:30 p.m. **Reception and Dinner**

Tuesday, June 5, 2018

Breakfast

Exhibitor Showcase

Welcome and Opening Remarks

- Dr. Robert DeOtte, West Texas A&M University

Welcome from the State of Texas

- Dr. T.R. Lansford, Region Director Texas Animal Health Commission

Welcome from the Region

- Dr. Brent W. Auvermann, Director Texas A&M AgriLife Research & Extension Center

Keynote Address: Mark Teachman, D.V.M., USDA

International Keynote:

Dr. Eran Raizman, Food and Agriculture Organization, United Nations

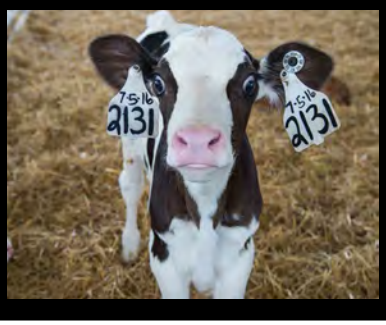
Technical Presentation Sessions:

Session 1: Optimizing Agriculture Systems/
Disposition of Final Products

Facilitator: Mary Schwartz, Cornell Waste Management Institute

- Optimizing Carcass Management Implementation Using Sensitivity Analyses from Risk Assessment - Lori P. Miller, USDA APHIS
- Animal Movement in Tunisia Using Social Network Analysis – Mohamed Naceur Baccar, National Center of Zoosanitary Vigilance
- Application of Diagnostic Tests to Inform the Choice of Disposal Option for Diseases Where Animals Can Recover - Sasidhar Malladi, University of Minnesota
- Vapor Phase Perioxide for Decontamination in Agriculture - Marek Kuzma, Institute of Microbiology





Tuesday, June 5, 2018 (Continued)

Session 2:
Emerging Disease Control and Environmental Impact

Facilitator: Mark King, Maine Department of Environmental Protection

- Carcass Disposal Methods during Major Epizootics: An Overview of African Swine Fever in Nigeria – Machebe S. Ndubuisi, University of Nigeria
- In-House Composting Field Exercise for Broiler Breeders - Gary Flory, Virginia Department of Environmental Quality
- Avian Influenza Mortality Management Options, Composting and Lessons Josh Payne, Jones-Hamilton

Livestock Mortality Management in Response to Natural Disasters

Facilitator: Bob DeOtte, West Texas A&M University

- Eric Glave, Kansas Department of Health and Environment
- T. R. Lansford III, DVM, Assistant Executive Director, Texas Animal Health Commission
- Mike Mayes – North Carolina Department of Agriculture and Consumer Affairs
- Jeremy Seiger – Oklahoma Department of Agriculture Food and Forestry

Technical Presentations:
Session 3: Federal, State and Industry Response

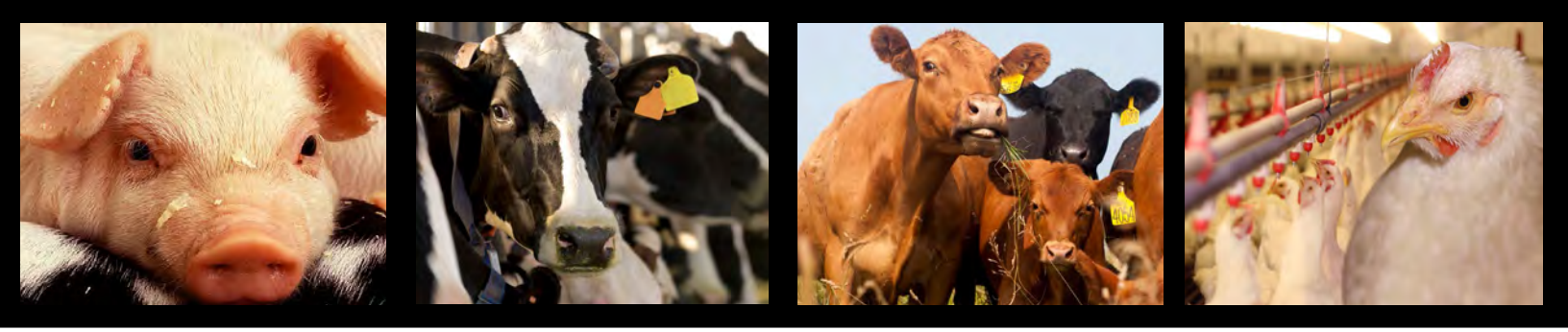
Facilitator: Dean Ross, Agrosecurity Consulting

- Federal 3D Priorities Update - Lori P. Miller, USDA APHIS
- Case Study of Enteric Illness in Responder Associated with 2015 HPAI Carcass Disposal Response - Lori Miller, USDA APHIS
- AVMA Humane Endings: An Update on the Panel on Euthanasia and the Panel on Depopulation - Cia Johnson, American Veterinary Medical Association
- Role of Meat Packing Industry in Response to Livestock Disasters Robert DeOtte, West Texas A&M

Session 4:
Emerging Disease Control and Environmental Impact

Facilitator: Zoe McManama, Wisconsin Department of Natural Resources

- Aquatic Disease Control of Infectious Salmon Anaemia in Atlantic Canada 2013- 2015 Sonya Natasha Piercey & Edward Malek, Canadian Food Inspection Agency
- Filth Fly Activity Associated with Composted and Non-Composted Beef Cadavers and Lab Studies on Volatile Organic Compounds - Justin Talley, Oklahoma State University
- Emergency Response: Composting in a Brucellosis Suis Outbreak Jean Bonhotal, Cornell University
- Livestock Carcass Disposal Exposure Assessments for Natural Disasters, Chemical, Biological and Radiological Emergencies - Sandip Chattopadhyay, U.S. Environmental Protection Agency



Tuesday, June 5, 2018 (Continued)

3D Meeting for 3D Committee Members

International Dinner for International Guests

Wednesday June 6, 2018

Technical Sessions:

Session 5: Carcass Management

Facilitator: Rebecca Podgorski, Wisconsin Department of Agriculture

- Burial Site Assessment, Construction, and Management for Environmental Protection and Disease Control - Zoe McManama, Wisconsin Department of Natural Resources
- Equine Carcass Composting – a Commercial Composting Model for Routine Mortalities - Michelle Melaragno, Kimberly Anne May, Compassionate Composting
- An Evaluation of the Efficacy of Composting as a Management Tool to Reduce the Viability of Newcastle Disease Virus – Mark A. King, Maine Department of Environmental Protection
- Recent Demonstration Projects and the Field Application of Aboveground Burial for Carcass Disposal - Gary Flory, Virginia Department of Environmental Quality

Session 6: Carcass Treatment

Facilitator: Ken Powell, Kansas Department of Health and Environment

- Ambient Alkaline Hydrolysis and Anaerobic Digestion for Management of Poultry Mortalities - Brandon H. Gilroyed, University of Guelph
- Rapid Mortality Disposal Using Containerized Composting - Jim McNelly Renewable Carbon Management LLC
- Efficacy and Efficiency of Poultry Carcass Composting Using Different Mechanical Mixing Equipment for AI Outbreaks - Jennifer Keaten, University of Iowa
- CO_2 Culling with Influenza Containment System I.C.S.: Physiological and Ethical Considerations -Abdelkader Alami and Bram Kamers, University of Lome

Global Issues in Animal Mortality Management:

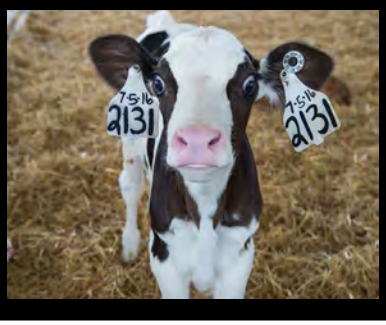
Challenges, Opportunities & Lessons Learned

Host: Gary Flory, Virginia Department of Environmental Quality

International Panelists:

- Dr. Eran Raizman, Food and Agriculture Organization of the United Nations, Italy
- Machebe Ndubuisi Samuel, PhD, University of Nigeria, Nigeria
- Mohamed Naceur Baccar, DVM, National Center of Zoosanitary Vigilance, Ministry of Agriculture, Tunisia
- Duncan Worsfold, Department of Economic Development, Jobs, Transport and Resources, Australia
- Edward Malek, Canadian Food Inspection Agency, Canada





Wednesday June 6, 2018 (Continued)

Sponsored by SCARAB International

Livestock Disaster Response Exercise Prep:
Continuity of Business Operations
Presenters:
- Robert DeOtte, West Texas A&M
- Ben Weinheimer, Texas Cattle Feeders Association
- Sandy Johnson, Kansas Department of Agriculture

Prepare for Departure for Demonstrations

Assemble for departure to the demonstrations

Concept and Equipment Demonstrations Bushland, Texas

Welcome: Dr. David Brauer, Director of USDA ARS Conservation & Production Research Laboratory / ARS and Texas A&M Agrilife Research and Extension Center, Bushland TX

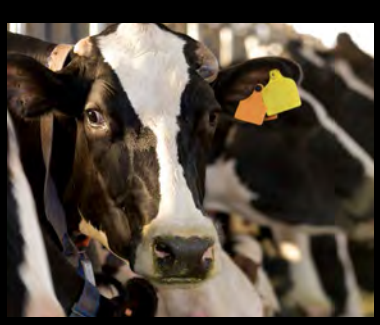
Return to hotel – Free Evening

Thursday, June 7, 2018

Emergency Exercise

Symposium Wrap-Up

Symposium Concluded

Educational Concept and Equipment Demonstrations

Aboveground Burial (AGB)—In this demo, aboveground burial is demonstrated with an adult Holstein cow placed on a 12-inch thick bed of carbon within an 18-inch deep trench. The animal is then covered with the soil that was excavated from the trench. Finally, the soil mound is seeded and the entire site is allowed to set while the carcass naturally decomposes. The decomposition process occurs in the shallow, biologically active soil zone where biological decay and the distance to groundwater is greatest. The AGB system was constructed during December 2017.

Deep Pit Burial—This is the traditional go to practice for most farm mortalities. In this case, a 6-8 foot deep pit is dug into the ground, with an optional liner added. The pit is just wide enough to accommodate a carcass, which is added and then the pit is back-filled. This demonstration was also built in December of 2017 and will be compared to the actions of the above ground burial. A viewing port has been added to allow carcass observations and leachate sampling is also included in this demo.

Soil Structure/Profile—In this demo an open soil pit will display the natural layers, or horizons, where the implications of soil texture and structure will be discussed as it relates to water movement and containment. Examples of regional soils will also be on display. Leachate movement through soil materials will be illustrated using soil columns.

Drone Monitoring—Fitted with an infrared temperature monitor, a commercial drone will be used to show how aerial monitoring might facilitate temperature monitoring of large mortality compost piles/ windrows during a foreign animal disease outbreak or natural disaster event.

Static Pile Compost –This demo is built in accordance with USDA Livestock Mortality Compost Guidelines and includes placing an adult Holstein carcass on a 12 long by 16 foot wide by 18 to 24-inch thick bed of carbonaceous amendment, followed by an additional 18-24 inches of carbonaceous material as a cover. The pile will be constructed in early April and will compost for 6-8 weeks prior to excavation.

Aerated Static Pile—This demo is set up similarly to the static pile demo except the base will be underlain by perforated PVC piping. This pile will also be built on the same day as the static pile and both will be compared for degree of degradation based on benefits of aeration.

Foam Euthanasia—In this demo, experts from North Carolina will show the proper techniques to humanely use foam to rapidly depopulate during a significant animal disease outbreak or other critical event. This demonstration will feature simulated animals only.



Alkaline Hydrolysis—During alkaline hydrolysis, mortalities are subjected to a process which rapidly speeds up decomposition using heat, pressure, and an alkaline substance such as potassium hydroxide or sodium hydroxide. Carcasses are loaded into a steel vessel with 80 gallons or so of water that is heated up to 300 degrees—killing any microbes and even destroying prions responsible for the Chronic Wasting Disease and Mad Cow Disease. After approximately two hours, most of the soft tissue is dissolved into a liquid and the remaining bone is brittle and can be easily ground up into ash.

Ask the Experts: In this tent, participants will have a chance to interact with 8 industry experts specializing in all aspects of mortality management.

- Becoming a Composting Subject Matter Expert (SME)
  Josh Payne – Oklahoma
- Efficacy and Efficiency of Poultry Carcass Composting Using Different Mechanical Mixing Equipment for AI Outbreaks
  Jennifer Keaton – Iowa
- Rapid Mortality Disposal Using Containerized Composting
  Jim McNelly – Minnesota
- Poultry Disposal after Hurricane Matthew in North Carolina
  Joe Hudyncia – North Carolina
- Informed Choice of Disposal Option for Diseases where Animals can Recover
  Sasidhar Malladi – Minnesota
- High Livestock Mortality Events in Kansas
  Erich Glave – Kansas
- Various Feedstocks Associated with Composting
  Bob Peer – Virginia

Outdoor Exhibitors:

Bock Industries
Bio-Response Solutions
Advanced Composting Technologies

Optimizing Carcass Management Implementation Using Sensitivity Analyses from Risk Assessment

Lori P. Miller, USDA APHIS

June 2018 | Amarillo, Texas

OPTIMIZING CARCASS MANAGEMENT IMPLEMENTATION USING SENSITIVITY ANALYSES FROM EXPOSURE ASSESSMENTS

LORI P. MILLER, PE
SENIOR STAFF OFFICER
U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
VETERINARY SERVICES
JUNE, 2018

Background

- Exposure Assessment of Livestock Carcass Management Options During Natural Disasters, (EPA, 2017) in collaboration with the U.S. Department of Homeland Security and the U.S. Department of Agriculture
- Exposure Assessment of Livestock Carcass Management Options During a Foreign Animal Disease Outbreak (EPA, 2017) in collaboration with the U.S. Department of Homeland Security and the U.S. Department of Agriculture
- Both documents are highly detailed and comprehensive assessments, publicly available in their entirety online; this presentation only focuses on the most significant points from the assessments in the presenter's opinion
- The base scenario assumes management of 50 tons of carcasses or (100) 1,000 lb cows, (565) 177 lb hogs, (25,000) 4 lb broiler chickens, or (5,000) 20 lb turkeys. Carcass management is assumed to take place at a hypothetical farm in Iowa.
- There are two types of contaminants of concern; chemical and microbial.

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Options Considered

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Offsite Options Controlled through Regulation – Risks not Evaluated

Tier 1 Ranking	Management Options	Chemical Exposure Pathways	Microbial Exposure Pathways	Controls and Limits to Environmental Releases
Rank 1: Negligible to minimal exposure — releases regulated to levels safe for human health and the environment	Incineration	6	6	Air emissions regulated under the Clean Air Act (CAA), including pollution control equipment (e.g., scrubbers, filters), with tall stacks to prevent localized deposition; residuals (i.e., ash) managed under the Resource Conservation and Recovery Act (RCRA); wastewater managed under the Clean Water Act (CWA).
	Rendering	3	2	Releases to air and to water regulated under the CAA and CWA, respectively.
	Landfilling	2	2	Landfill design and operation regulated under RCRA; controls include leachate collection and management and methane recovery.

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Most Significant Exposure Pathways for Livestock Carcass Management - Chemicals

- Storage pile leaching to groundwater and surface water/fish ingested by humans/livestock

- Burning air inhalation, deposition on crops and deposition on surface water to fish consumed by humans
- Leaching from ash burial to groundwater ingested by humans/livestock

- Deep burial leaching to groundwater and surface water to fish ingested by humans/livestock

- Compost pile leaching to groundwater and surface water to fish ingested by humans
- Land applied compost leaching to groundwater and taken up by crops ingested by humans

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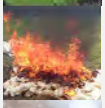
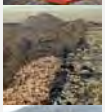
Chemical Ranking Ratio Summary

- Storage Pile – top risks from Fe and Zn ingestion (median 3.9E-10)

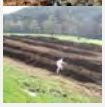


- Open Burning – top risks from Mn and Ni inhalation (median 4.0E-02)

- Air Curtain – top risks from Mn and Cr inhalation (median 2.0E-02)



- Deep Burial – top risks from Fe and Zn ingestion (median 6.3E-09)



- Compost Windrow – top risks from Fe and Zn ingestion (median 3.1E-10)
- Compost Application – top risks from Fe and Cr ingestion (median 5.1E-02) can be mitigated with erosion control measures

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Ranking of Onsite Options for Chemicals

Rank [a]	Management Option	Principal Rationale
1	Compost Windrow	Bulking material retains most chemicals
1	Burial	Soils filter out chemicals traveling toward groundwater
2	Air-curtain burning	Similar release profiles; emissions sensitive to type and quantity of fuels used and burn temperature
2	Open Pyre burning	
3	Compost Application	If no offset from lake; mitigate with offset and erosion controls

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Most Significant Exposure Pathways for Livestock Carcass Management – Naturally Occurring Microbes

- Storage pile leaching to groundwater ingested by humans/livestock

- Burning leaching from ash burial to groundwater ingested by humans/livestock

- Deep burial leaching to groundwater ingested by humans/livestock

- Compost pile leaching to groundwater ingested by humans/livestock
- Land applied compost leaching to groundwater and taken up by crops ingested by humans/livestock

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Ranking On-site Carcass Management Options by Relative Risk from Microbes

	Carcass Management Option	Rationale
1	Air-curtain burning	All microbes inactivated or destroyed, lowest relative risk
2	Open-pyre burning	Prions survive, other microbes inactivated or destroyed
3	Composting: windrow & application	Prions and spores survive, *E. coli* can be inactivated
4	Burial	No thermal destruction; leachate not impeded

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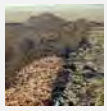
Most Significant Exposure Pathways for Livestock Carcass Management – FAD Pathogens

- Storage pile
  - air inhalation
  - air deposition on plants ingested by humans/livestock
  - air deposition on soil and surface water incidentally ingested by humans/livestock
  - leaching to groundwater ingested by humans/livestock



- Deep burial leaching to groundwater ingested by humans/livestock

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Ranking of Onsite Options for FAD Pathogens

Rank	Management Type	Principal Rationale
1	Open Burning and Air-curtain Burning	Thermal descruction of all FMDv.
2	Composting	Bulking material contains almost all FMDv from releases to air and soil. Thermal inactivation and biological decay eliminate FMDv before composting is complete.
3	Burial	Cover soil contains releases to air. If a number of conditions are met, leaching has the potential to infect cattle that drink water pumped from a ground water well.
4	Temporary Storage	Cattle can be infected by inhaling or ingesting FMDv emitted to air from a nearby storage pile. If a number of conditions are met, leaching has the potential to infect cattle that drink water pumped from a ground water well.

FMDv = foot and mouth disease virus

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Livestock FMD Exposure Pathways

Exposure Source	Carcass Management Options	
	Temporary Storage Pile	Burial
Air Inhalation	1) Air[a]	1) Air[b]
Direct Ingestion	2) Air → Plants[a]	2) Air → Plants[b]
Incidental Ingestion	3) Air → Soil[a]	3) Air → Soil[b]
Incidental Ingestion	4) Air → Surface water[a]	4) Air → Surface water[a]
Ground-water Ingestion	5) Leachate → Ground water[a]	5) Leachate → Ground water[a]
Vectorborne Transmission	6) Airborne vectors → Livestock[c]	6) Airborne vectors → Livestock[b,c]

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Variables and Effects on Exposure

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Scale of Mortality

RELEASES/EXPOSURES

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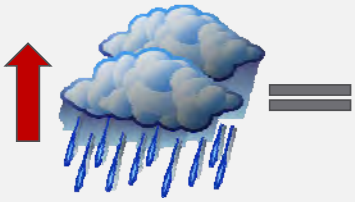
Meteorology



RELEASES/EXPOSURES

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Soil Particle Size and Type

RELEASES/EXPOSURES

Table 3-2. Average Downward Travel Velocities and Time to 1 m Depth in Unsaturated Soils

Soil Type	Average Downward Water Velocity (cm/day)	Average Time to Breakthrough (day)
Sand	100[a]	1.0
Loam	18[b]	5.6
Clay	2.5[c]	40

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Soil Organic Content

MICROBIAL RELEASES/ EXPOSURES

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Surface Slope

Faster and farther surface movement of leachate from storage piles

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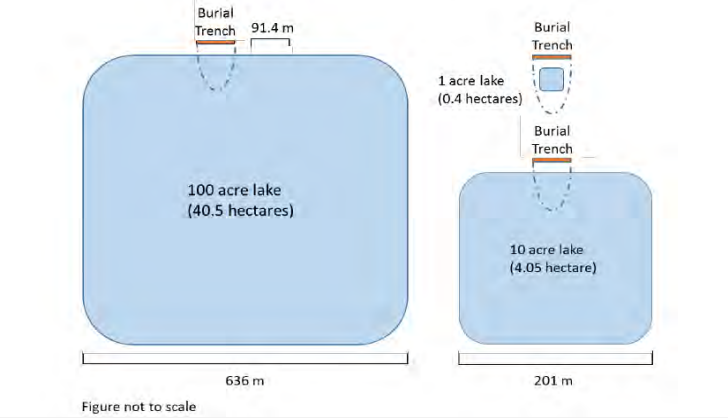
Relationship Between Burial Trench Groundwater Plume and Lakes of Various Sizes



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Home-Grown Foods

Potential chemical and microbial exposures

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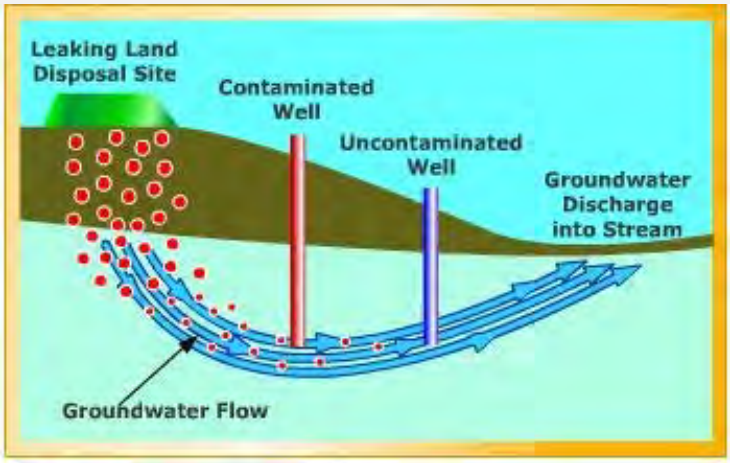
Groundwater Hydrology



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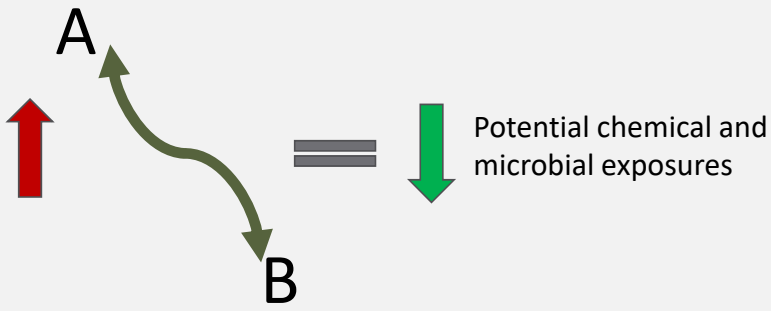
Distance from Source



Potential chemical and microbial exposures

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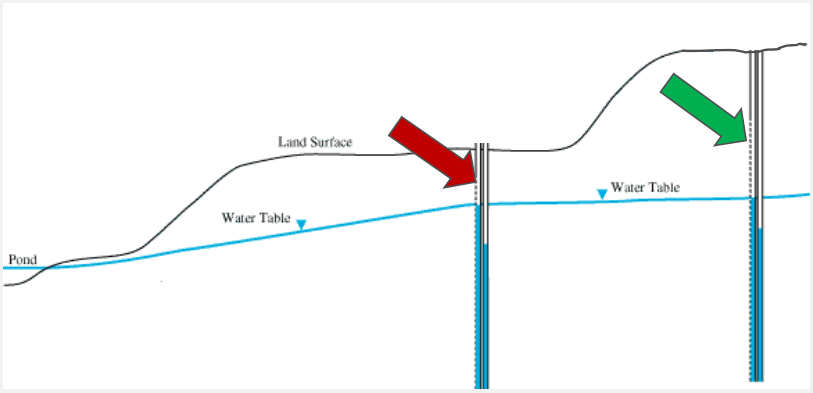
Depth to Groundwater



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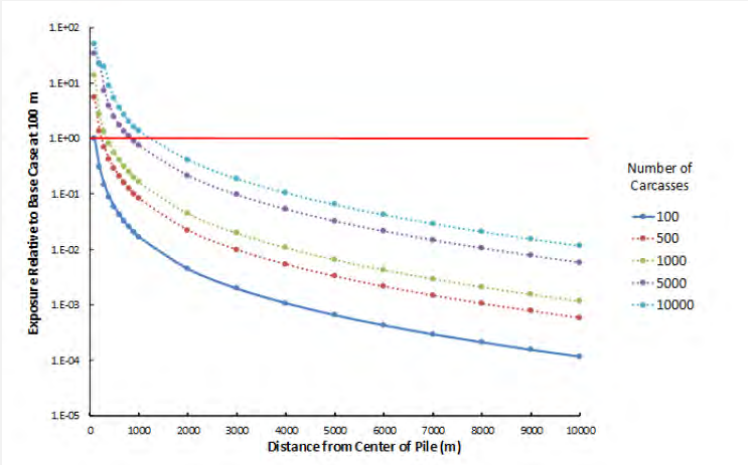
Uncertainty analysis for the number of carcasses, inhalation exposure for dairy cattle relative to the base case, with distance from the storage pile.



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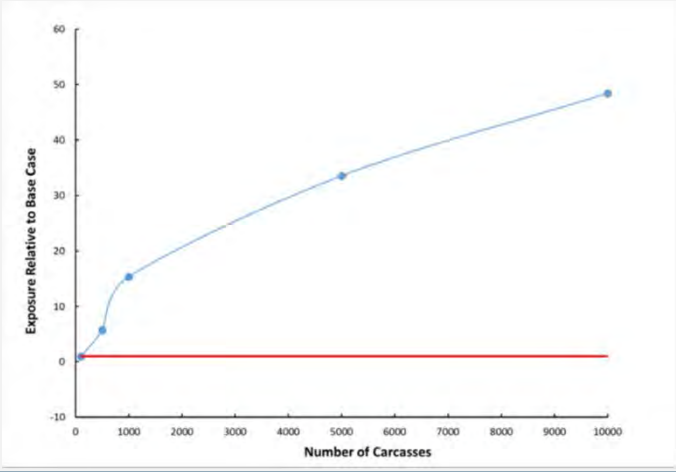
Uncertainty analysis for number of carcasses, ingestion exposure for dairy cattle relative to the base case at 100 m from the storage pile.



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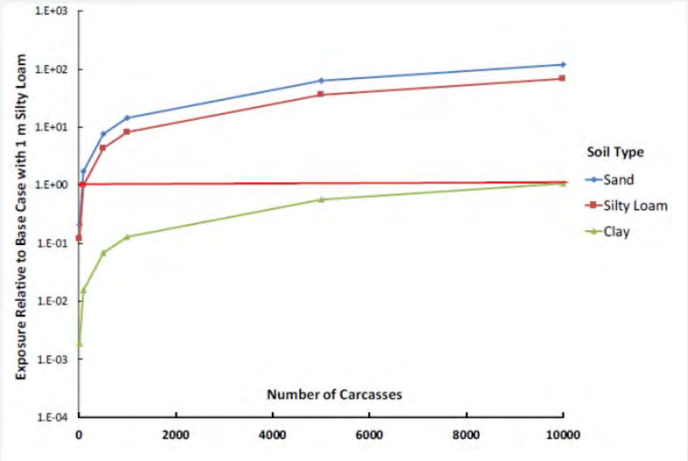
Uncertainty analysis for the number of carcasses, water ingestion exposure for dairy cattle by soil depth, relative to exposure with 100 carcasses and silty loam.



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Mitigations

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On-Site Combustion Mitigations

- Install units downwind or at least 1,000 m upwind from homes, businesses, farm buildings, crops, pastures, and surface waters
- Monitor burn piles to maintain even heating over time, and ample ratio of fuel to carcasses.
- Landfill ash or bury/encapsulate with clean soil.
- Isolate ash from root zone of plants.
- Wet the ash prior to burial, and minimize handling and processing.
- Do not use the ash as a surface soil amendment.

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On-Site Burial Mitigations

- Place burial sites down-gradient of groundwater wells or surface water bodies;
- Comply with required setback distances and other site restrictions.
- Comply with minimum requirements for depth above the water table.
- Properly lime the carcasses if required by the jurisdiction.
- If feasible, include a liner of compacted clay in the bottom of the burial trench.
- Install ventilation shafts to release gas pressure and protect cover soil.
- Restrict access or minimize activity at the site to protect cover soil.
- Monitor and maintain cover soil over time

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On-Site Composting Mitigations

- Maintain required temperature and time standards
- Use required quantity and quality of carbonaceous material
- Use required depth of cover and base material
- Test soil under windrow for chemical levels before growing food or allowing grazing.
- Leave required buffer distance between windrow and surface and ground water
- When land applying finished compost, prevent runoff to surface water; revegetate immediately

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Off-Site Management Mitigations

- Maintain strict biosecurity at off-site facilities
- If rendering, ensure meat and bone meal is not used for animal feed if prions may be present

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Carcass Handling Mitigations

- Do not handle carcasses with bare hands, especially if there are visible signs of bloating/leakage
- Use appropriate personal protective equipment based on a comprehensive job hazard analysis conducted by a qualified safety professional

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Temporary Carcass Storage Mitigations

- Locate storage pile on impervious surface or liner
- Contain leakage and run-off
- Cover storage pile
- If storage indoors, provide adequate ventilation

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Carcass Transport Mitigations

- Use leak-resistant vehicles and liners with absorbent material
- Cover load with secure tarp
- Load vehicles less than 60% full by volume
- Transport loads immediately

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Application of Diagnostic Tests to Inform the Choice of Disposal Option for Diseases Where Animals Can Recover

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June 2018 | Amarillo, Texas

Application of Diagnostic Tests to Inform Disposal Option Choice for Diseases Where Animals Can Recover

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Written for presentation at the
6th International Symposium on Animal Mortality Management
Amarillo, Texas
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Abstract. *Early depopulation and disposal have been recognized as key factors in the control of animal disease outbreaks. However, there are additional considerations when managing outbreaks of diseases where animals may recover from an acute viral infection. In this case, the proportion of animals that are actively shedding virus would be lower at later stages of disease spread in the population when most of the animals would have recovered. We discuss how the prevalence of infectious and recovered birds varies over time, and how this information can be used to guide decisions regarding off-site disposal options, based on an example scenario of low pathogenicity avian influenza (LPAI) infection in a broiler-breeder flock. First, we used stochastic simulation models to predict the proportion of infectious and recovered birds over time in an LPAI infected broiler-breeder flock. We then simulated detection using various diagnostic testing options, including serological testing of 15 samples using the Agar Gel Immunodiffusion (AGID) assay, and an influenza A matrix-gene real-time reversed transcriptase polymerase chain reaction (RRT-PCR) testing protocol using pooled samples of 11 swabs each, and combinations of RRT-PCR and AGID. The simulation models were used to predict the range of time to detect LPAI post exposure and the proportion of infectious birds in the flock at the time of detection under various active surveillance protocol options. We then used the simulation model results to show the benefit of additional diagnostic testing in reducing uncertainty in the outcome variables and providing confidence that the number of infectious birds at the time of movement to disposal are likely to be very low (acceptable). Our results indicate that a combination of RRT-PCR and AGID provides the most information regarding the prevalence of infectious birds and the additional number of days required for the flock to stop shedding. Finally, we discuss how the concepts and approach illustrated through the LPAI example may be generalized to other diseases where the animal populations would eventually recover.*

Keywords. Disposal, Low Pathogenicity Avian Influenza, Active Surveillance, Agar Gel Immunodiffusion

Introduction

Timely access to multiple carcass disposal options is important for the management of animal disease outbreaks or other mass mortality events. The limiting factors impacting the choice of a disposal option include logistical feasibility, environmental considerations, disposal costs, and risks associated with handling infectious animal waste. While off-site disposal options such as rendering may have potential economic benefits (e.g., reducing downtime before production can be resumed, partial compensation to offset disposal costs, etc.), off-site disposal may also be perceived to be a higher risk for disease transmission.

Early detection and rapid depopulation are common strategies used to manage outbreaks of highly contagious foreign animal diseases. These strategies are well suited for outbreaks of highly pathogenic avian influenza (HPAI) in commercial poultry, which typically causes acute clinical signs, including mortality. In this case, dead-bird targeted active surveillance has been shown to reduce time to detection in infected flocks, ensuring a lower prevalence of infectious birds at the time of depopulation and disposal, and thus reducing the potential risk of further disease spread. However, there are additional considerations when managing outbreaks of diseases where animals may recover from an acute viral infection. In the case of low pathogenicity avian influenza (LPAI), detection would likely occur at a later stage of disease progression in the flock because of milder clinical signs and much lower disease mortality. Moreover, the prevalence of infectious birds would be much lower at later stages of disease spread in the flock, as most birds would have recovered. The potential risk of disease spread associated with off-site disposal would also be correspondingly lower during the later stages of infection, given the lower prevalence of actively shedding birds.

Therefore, additional disposal options may be considered in situations where it can be determined that most of the birds have recovered, and are not actively shedding virus. Results from active surveillance testing using different types of diagnostic tests can be used to predict the prevalence of infectious birds in the flock, and the time until most birds in the flock stop shedding.

As an example, we consider the case of an LPAI infected broiler-breeder flock, detected through routine surveillance testing for H5/H7 LPAI. First, we used a within-flock disease transmission model to predict the prevalence of infectious and recovered birds over time. Next, we simulated detection via various diagnostic testing options[1] that include; 1) serological testing of 15 samples using the Agar Gel Immunodiffusion (AGID) assay, and 2) influenza A matrix-gene real-time reversed transcriptase polymerase chain reaction (RRT-PCR) testing using pooled samples of 11 swabs. We use the simulation model results to show the benefit of additional diagnostic testing to reduce uncertainty in outcome variables, providing confidence that the number of infectious birds at the time of movement to disposal would be very low. We discuss how the prevalence of infectious and recovered birds varies over time, and how this information can be used to guide decisions regarding off-site disposal options, based on an example scenario of low pathogenicity avian influenza (LPAI) infection in a broiler-breeder flock.

Materials and Methods

[1] We used these surveillance options as examples, in an effort to be consistent with current NPIP minimum requirements outlined in 9 CFR 145.33(l) U.S. Avian Influenza Clean. As applied to breeding, a flock is all poultry of one kind of mating and of one classification on one farm. Therefore, if 15 samples are required, 1 pool of 11 swabs for antigen testing would meet these minimum standards.

We evaluated two routine (i.e., in the absence of an outbreak) surveillance-testing protocol options (Options A and B) for detecting LPAI in broiler-breeder flocks.

Protocol Option A: 15 blood samples taken from live birds in one flock and tested via AGID at 90 day intervals.

Protocol Option B: One 11-swab pooled sample per-flock is tested via RRT-PCR at 90 day intervals. The available dead birds are sampled first, followed by live birds as necessary to get a total of 11 swabs.

We used stochastic simulation models to predict the prevalence of infectious, dead, and recovered birds over time in an LPAI infected broiler-breeder flock. Some of the models used are adaptations of those described in earlier work (Weaver et al., 2015). The transmission model output was then used to simulate detection via active surveillance, and to predict the time post-exposure when LPAI is detected. Details of these procedures are as follows:

Protocol Option A used the transmission model output for both the number of live and seropositive birds at the time of sampling. The number of samples from seropositive birds was first simulated based on the flock sero-prevalence at the time of sampling and testing. The number of positive serology results from tests on seropositive bird samples was then simulated using a binomial distribution, with probability of detection equal to the serological test sensitivity. If at least one of the results was positive, LPAI was detected in the flock.

Under Protocol Option B, the proportion of infectious birds actively shedding virus in the sample pool was simulated from the transmission model-predicted disease mortality and normal daily mortality data from 8 broiler-breeder flocks. Given a virus-positive sample, the detection process via RRT-PCR testing was simulated as a Bernoulli trial, with the probability of success equal to the RRT-PCR test sensitivity.

In addition to detection, diagnostic test results are useful in establishing the stage of LPAI disease progression, the proportion of infected birds actively shedding virus, and the number of days for the flock to stop shedding virus. We evaluated the benefit of diagnostic testing's ability to provide information on these relevant epidemiological outcomes. We predicted the proportion of infectious birds actively shedding virus, and the time to stop shedding virus, conditional on the number of positive results from 15 blood samples tested via AGID. We performed 100,000 iterations of the simulation model implemented in the software R (R Core Team, 2015) and extracted the simulations where the specific test results were obtained. The distributions of the relevant epidemiological variables conditional on the test results were then estimated from the extracted simulations.

One critical input in the disease transmission simulation model is the adequate contact rate, which determines the rate of within-flock infection spread. There is considerable uncertainty regarding this parameter for LPAI spread in chickens, given the limited number of estimates from outbreak data in the published literature. To address the uncertainty associated with this parameter, we used two scenarios for the adequate contact rate. In the slow contact rate scenario, we used Uniform (0.69 - 0.77) per-day as the adequate contact rate distribution based on the estimates for LPAI infected cage-free egg-layer flocks in the Netherlands (Gonzales et al., 2012). We also evaluated a fast contact rate scenario using contact rate estimates from HPAI infected flocks (Uniform (2.68 - 7.57) per-day), where field data was available from a greater number of outbreak flocks (Bos et al., 2009). The parameters of the disease transmission and surveillance simulation models are summarized in **Table 1**. The test-day was allowed to vary from 1 to 40 days and 1 to 65 days post-exposure of the flock in the faster and slower LPAI within-flock disease spread scenarios respectively (i.e., it would take much longer for LPAI infection to spread through the entire flock in the slower spread scenario).

Results

The model-predicted time post-exposure (days) given detection of LPAI in a broiler-breeder flock under routine surveillance at 90 day intervals under the slow spread scenario is shown in **Figure 1**. Mean post-exposure time given that 1 pool of 11 swabs tested positive by RRT-PCR was predicted to be 22.97 (90% Prediction Interval (P.I.) 11 to 30) days, and 41 (90% P.I. 19 to 62.5) days when at least one of 15 samples was positive using AGID. The relatively wider interval for time post-exposure for detection via AGID is because detection could occur over a wide range of possible times, including days when the flock has completely recovered.

To address uncertainty in the rate of disease spread on the range of possible outcomes, results are presented for faster and slower within-flock disease transmission rates. **Figure 2** shows the predicted number of days until all infectious birds stop shedding in an LPAI infected broiler-breeder flock, given results from a set (from 0 to 15 out of 15) of AGID serological test results. In the case of faster within-flock LPAI virus spread (top panel), it took fewer days for the flock to stop shedding given a set of test results.

Results for the percentage of infectious birds in an LPAI infected flock, and the number of days until all birds in the flock stop shedding, given a set of serological test results (AGID), are provided in **Table 2**. Results are interpreted from the perspective that the flock is known to be infected. In the event that there are no positive serological test results out of 15 samples submitted, our model predicts that 8 percent (90% P.I., 0 to 48 percent) of birds in the flock could be shedding virus at that time, and it may take 34.5 days (90% P.I. 25.5 to 43.75 days) on average for all birds in the flock to stop shedding virus. In the event that all 15 serological samples return positive test results, 1 percent (90% P.I. 0 to 6 percent) of the birds in the flock could be shedding virus, where only 2.9 days (0 to 13.25 days) on average are required to complete shedding. As the number of positive serological test results increase, our uncertainty about the level of shedding in flock decreases (i.e., the width of the 90th percentile prediction interval decreases considerably).

Discussion

Timely access to multiple carcass disposal options is important for the management of animal disease outbreaks. However, some off-site disposal options such as rendering, burial in municipal solid waste landfills, or movement to slaughter may be perceived to be a higher risk for disease transmission because of the movement of potentially infectious animals. For diseases where the animals can eventually recover, the proportion of animals that are actively shedding virus would be low in the later stage of diseases progression, when most of the animals would have recovered. For commercial poultry flocks infected with LPAI virus, additional disposal options may be considered in situations where it can be determined that most of the birds have recovered, and are not actively shedding virus. Results from active surveillance testing using different types of diagnostic tests can be used to predict the proportion of birds in the flock actively shedding virus, and the time until most birds in the flock stop shedding. We illustrated these concepts for recoverable diseases based on the case of an LPAI infected broiler-breeder flock, detected through routine surveillance testing for H5/H7 LPAI.

The time interval from detection to when most of the birds have recovered and stopped shedding virus is a key consideration when choosing a disposal option. Our results indicate that LPAI infected flocks may be detected at a later stage of disease progression under routine H5/H7 surveillance protocols used to test broiler-breeder flocks in the United States, given their typically mild clinical signs. In particular, when using serological tests alone, there is a higher likelihood of a detected flock being in a later stage of infection when most of the birds have

recovered. Therefore, for some LPAI infected broiler-breeder flocks detected via routine surveillance, it is possible that the time until all birds in the flock would stop shedding would not be very long.

The results from our analysis indicate that diagnostic test results could be useful when making decisions on a disposal option by reducing the uncertainty in the current stage of infection (i.e., actively shedding versus mostly recovered), and the number of days required for the flock to stop shedding. For instance, positive test results from all 15 serological samples would indicate that the flock has stopped shedding or is likely to stop shedding within a few days. Conversely, 1 or 2 positive serological test results out of 15 samples would indicate that it may take considerably longer for the flock to stop shedding. Therefore, a set of diagnostic test results can provide valuable information, and can be used to partly inform risk management decisions pertaining to the choice and timing of disposal options, along with other key considerations.

Each disposal option has specific advantages and disadvantages. Having access to multiple disposal options during an outbreak provides flexibility for emergency responders. Moving carcasses off-site to a landfill for disposal, for off-site burial, or for rendering may reduce the time needed for an infected premises to resume production, compared within-house composting. Disposal of infected flocks by rendering could potentially increase revenue for the producer and reduce disposal costs, although further economic analysis is needed to address this aspect. Based on the current analysis, waiting until the flock has stopped shedding, based on diagnostic testing, can help make off-site disposal options tenable. However, when choosing a disposal option, risk managers should also consider the additional risk of local spread. This includes spread from the premises during any waiting period, as well as the risk of spread during transportation associated with contamination of equipment and fomites used to move live or dead birds.

The approaches and concepts proposed as options for disposal of LPAI infected flocks put forth in the current analysis can also be generalized for other disease agent-host combinations where animals can recover from infection. Further research and economic analysis of the costs, benefits, and potential economic consequences of various off-site disposal options for flocks or herds affected by recoverable diseases, where decisions on waiting-time and off-site movement are informed based on diagnostic testing, would benefit risk managers who are dealing with animal disease outbreaks.

Table 1. Summary of disease transmission and active surveillance model parameters.

Parameter Name/Notation	Description	Distribution/Value
Adequate contact rate	Mean number of contacts per day each bird has with other birds such that the contact is sufficient to transmit infection	Slow rate ~ Uniform (0.69 - 0.77); Fast rate ~ Uniform (2.68 - 7.57)
Flock size	Number of birds in a broiler breeder house	~ Uniform (9000, 10000)
Latent period distribution	Length in days of the latent period	~ Gamma (shape = 0.82, scale = 0.44)
Infectious period distribution	Length in days of the infectious period	~ Gamma (shape = 8.14, scale = 0.96)

Time to seroconversion distribution	Length in days of time from infection to seroconversion	~ Gamma (shape = 10.03, scale = 0.63)
p_{mort}	Proportion of birds that die following exposure to LPAI	0.005
p_{sero}	Proportion of birds that seroconvert following exposure to LPAI	0.95
Se_{pcr}	rRT-PCR test sensitivity	0.865
Se_{sero}	Serology test sensitivity	0.95

Table 2. The percentage of infectious birds in an LPAI infected flock, and the number of days until all birds in the flock stop shedding, given a set of serological test results (AGID) out of 15 samples submitted. Results are for a slower within-flock disease spread rate.

Number of positive serological test results out of 15 samples tested	**Days until all birds stop shedding LPAI virus**	**Proportion of birds in the flock shedding LPAI virus**
0	34.5 (25.50 – 43.75)	0.08 (0 – 0.48)
1	26.1 (21.50 – 32.00)	0.5 (0.09 – 0.76)
2	24.5 (20.00 – 29.74)	0.65 (0.36 – 0.77)
3	23.4 (19.25 – 28.25)	0.71 (0.59 – 0.77)
4	22.6 (18.36 –27.75)	0.72 (0.6 – 0.77)
5	21.4 (17.80 – 26.20)	0.69 (0.55 – 0.77)
6	21.1 (17.15 – 25.75)	0.66 (0.48 – 0.76)
7	20.3 (16.25 – 25.75)	0.6 (0.43 – 0.74)
8	19.5 (14.75 – 25.31)	0.54 (0.33 – 0.71)
9	17.4 (1.26 – 23.74)	0.43 (0 – 0.69)
10	12.7 (0 – 22.00)	0.26 (0 – 0.61)
11	8.5 (0 – 20.75)	0.13 (0 – 0.52)
12	5.4 (0 – 18.00)	0.05 (0 – 0.33)
13	4 (0 – 15.75)	0.02 (0 – 0.19)
14	3.4 (0 – 14.25)	0.01 (0 – 0.1)
15	2.9 (0 –13.25)	0.01 (0 – 0.06)

[a] Mean and 90th percentile prediction interval.

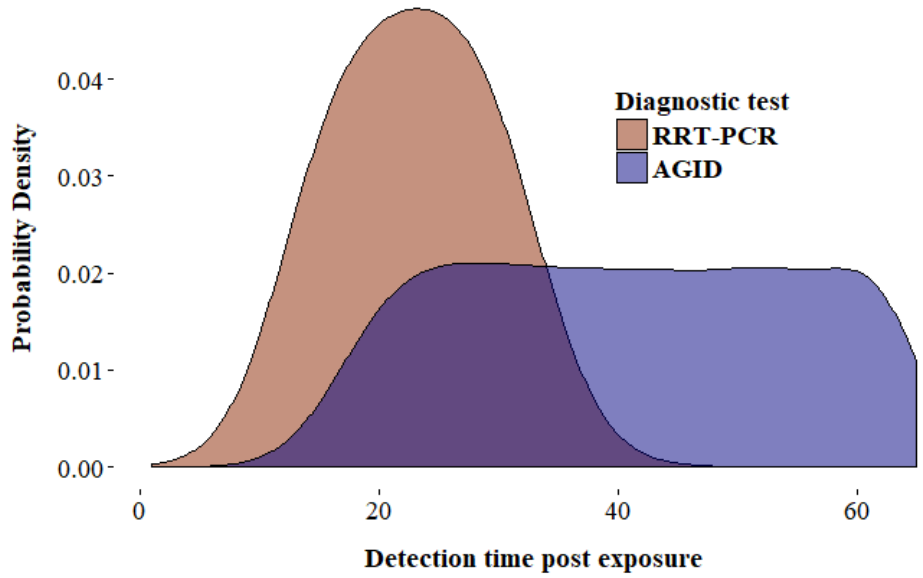

Figure 1. Time post-exposure (days) given detection LPAI in a broiler-breeder flock using either RRT-PCR (1 pool of 11 swabs per-flock) or AGID. Mean detection time post-exposure for RRT-PCR was 22.97 (90% P.I. 11 to 30) days, and 41 (90% P.I. 19 to 62.5) days for AGID. Results are for a slower within-flock disease spread rate.

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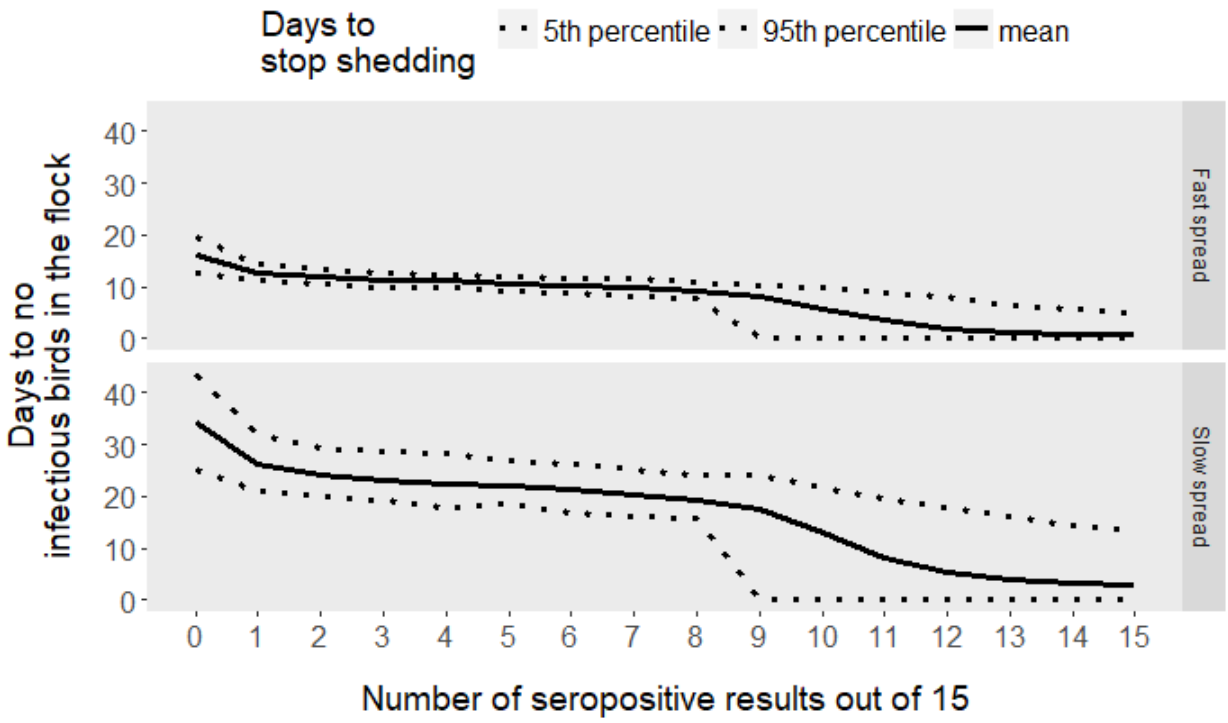

Figure 2. The predicted number of days after test-day until all infectious birds stop shedding LPAI virus in an infected broiler-breeder flock, given results from a set (from 0 to 15 out of 15) of AGID serological test results. The top panel represents faster LPAI spread within a flock, and the lower panel represents slower rates of spread.

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Carcass Disposal Methods during Major Epizootics: An overview of African Swine Fever in Nigeria

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June 2018 | Amarillo, Texas

Carcass Disposal Methods during Major Epizootics: An Overview of African Swine Fever In Nigeria

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Written for presentation at the
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Abstract. Increased globalization, changes in livestock production systems, decline in animal health services and infrastructure especially in developing countries and global warming are factors that have contributed to the dynamic nature of transboundry animal diseases. In Nigeria, the management of livestock is also becoming increasingly difficult due to economic recession. Where these factors are not properly managed, a major epizootic may result. When there is a major animal disease outbreak, along with the need for immediate disease containment, a very significant question that relates to the method of handling potentially large number of dead animals will definitely arise. If stamping out method of disease control, the most common and successful approach particularly in exotic diseases such as Highly Pathogenic Avian Influenza or African Swine Fever (ASF) is chosen, the method of animal carcass disposal for slaughtered animals must also be decided. There are, also apart from disease outbreaks, many situations that may also lead to the death of animals in large numbers. These include natural disasters such as flooding or hurricanes, animal contamination by toxic chemical spills, ingestion of contaminated feed, large fires, and slaughter for animal welfare reasons such as starvation, humane culling, or deliberate bioterrorism. Although these situations could take advantage of the same planning strategy, in Nigeria, many farmers neither have any knowledge of the existence of regulatory control on carcass disposal nor has any been prosecuted for improper carcass disposal. This paper therefore examined methods of carcass disposal in Nigeria during major epizootics such as African swine fever.

Keywords. African swine Fever, mortality, carcass disposal, disease, livestock

Introduction

According to Beltrán-Alcrudo *et al.* (2017), within global livestock production, the pig sector plays a key role as a source of animal protein. Largely due to the increase in worldwide demand for meat, pigs have become a crucial food source due to their fast growth, efficient feed conversion, quick turnover, and prolificacy. Pork is the most consumed meat from terrestrial animals, accounting for over 37 percent of global meat intake, followed closely by chicken 35.2 percent and beef 21.6 percent (FAO, 2013). The pig sector has grown steadily over the past decades but the increase has been uneven around the globe. Large populations occur in China and parts of Southeast Asia such as Viet Nam, in Western Europe, Central and Eastern areas of the United States, Central America, and Southern Brazil (FAO, 2017). The sector is characterized by a deep divide between traditional, small-scale, subsistence productions and industrialized pig farming with increasing vertical integration. These two very different stakeholder groups have different priorities in adjusting production practices or investing in biosecurity to prevent and control pig diseases. Indeed, the backyard sector, characterized by low biosecurity, outdated husbandry practices and technologies, and poor awareness of, and compliance with, animal health regulations (outbreak reporting, movement control, certifications, vaccination, etc.) plays a major role in the introduction, spread, and maintenance of ASF and several other pig diseases(Robinson *et al.*, 2011; FAO, 2017).

African Swine Fever

African Swine Fever (ASF) is a highly contagious viral disease of domestic pigs; it manifests itself as a haemorrhagic fever and results in up to 100 per cent mortality (Fig: 1). The causative agent of ASF is a unique, enveloped, cytoplasmic, double-stranded DNA arbovirus, which is the sole member of the family *Asfarviridae*. Although it was generally considered that there is only one serotype of ASF virus, recent studies have reported the classification of 32 ASFV isolates in eight different serogroups based on a haemadsorption inhibition assay (Malogolovkin*et al.*, 2015).

The catastrophic effect of this disease on pig production, from household to commercial level, has serious socioeconomic consequences and implications for food security. It is a serious transboundary animal disease with the potential for rapid international spread (FAO, 2001).

Fig 1: Pig mortality during ASF incursion.

World Distribution of African Swine Fever

The disease was first described by Montgomery in 1921 in Kenya, ASF has subsequently been reported from most countries in southern and eastern Africa, where the virus is maintained either in a sylvatic cycle between warthogs (*Phacochoerusaethiopicus*) and ticks of the *Ornithodorosmoubata* complex or in a domestic cycle that involves pigs of local breeds, with or without tick involvement (FAO, 2001). Countries where endemicity is confined to the sylvatic cycle include Kenya, Namibia, Botswana, Zimbabwe and northern South Africa. A cycle in

domestic pigs apparently occurs in Angola, the Democratic Republic of the Congo, Uganda, Zambia, Malawi, Northern Mozambique and probably the Congo (Brazzaville), Rwanda, Burundi and Tanzania. Madagascar experienced ASF for the first time in 1997–98; it caused serious losses and has not yet been eradicated (Plowright *et al.,* 1994).

The disease spread to Portugal in 1957, almost certainly from Angola. Although it was apparently eradicated, a second introduction in 1959 resulted in spread throughout the Iberian Peninsula and to several other countries in Europe, including France, Italy, Malta, Belgium and the Netherlands. It became well established in Spain and Portugal, where eradication was only accomplished in the early 1990s and remains endemic on the Italian Island of Sardinia. Portugal experienced an outbreak in late 1999, which was evidently rapidly contained.

In 1977, ASF spread to Cuba, where it was eradicated with the loss of some 400 000 pigs. Outbreaks occurred in Brazil and the Dominican Republic in 1978, Haiti in 1979 and Cuba in 1980.Eradication from these countries was achieved only by massive depopulation of pigs. Whether these outbreaks originated in Europe or Africa has never been established. African swine fever has been endemic in Cameroon since the first reported outbreaks in 1982.

In West Africa, it has been endemic in Southern Senegal, the Gambia and probably Guinea Bissau and the islands of Santiago and Mao in the Republic of Cape Verde. The disease has been present in this focus since at least 1958–60. An outbreak of ASF occurred in Nigeria in 1973. In 1996, Côte d'Ivoire experienced a massive outbreak that spread rapidly through the southern parts of the country. The last focus was extinguished by October 1996. In October 1997, ASF was reported in Benin, rapidly followed by Togo and two western provinces of Nigeria. Spread in these countries was rapid. In October 1999, ASF was reported in Ghana. Rapid implementation of control measures has apparently been successful, as no ASF has occurred since February 2000. Because of civil unrest in various regions and lack of disease reporting from some countries, the ASF status of a number of countries in Africa is unknown (FAOSTAT – http://www.fao.org/faostat/). All of the countries in sub-Saharan Africa that have significant pig populations must be considered to be infected, potentially infected or at risk from ASF.

In Nigeria, an outbreak of ASF occurred in 1973 in a piggery in Abeokuta, Ogun State where all the 3000 pigs in the farm died from the disease. In October 1997, ASF was reported in Benin, rapidly followed by Togo and in September 1997 the disease surfaced in free-ranging pigs in four local government areas of Ogun State, of Nigeria that have common borders with Benin Republic. The disease was first seen in villages alongside the lagoon passing into Nigeria from Benin Republic (FAO, 1998). According to Obi (2014), dead pig carcasses were seen in the lagoon and there was evidence that boats were traveling along the lagoon selling pig meat in Badagry Market and nearby villages. By December 1997 ASF was reported in Badagry in Lagos State, Nigeria and from the Lagos and Ogun State foci, the disease eventually spread to Osun, Oyo, Ondo, Ekiti, Edo, Delta, Anambra, Enugu, Abia, Rivers, Bayelsa, Akwa-Ibom, Cross-River, Benue, Kaduna and Plateau States of Nigeria. By October 1998 about 125,000 pigs had died of the disease in nine states resulting in estimated loss of N1.0 billion. In Benue State which accounts for about 21% of the national swine herd, 3,108 pig farmers in 20 out of the 23 Local Government Areas of the State were affected and 78 per cent of the 98,443

affected pigs died of ASF at an estimated financial loss of ₦ 335,954,000 ($2,777,777.78). According to Obi (2014), the national average monetary loss per pig rearing household was estimated at ₦ 55,655 ($154.60).

Apart from the immense financial losses from ASF, the outbreaks led to lack of capital for restocking, loss of confidence by pig farmers in the profitability of pig production as well as had demoralizing effects on pig marketers, loaders and pig processing enterprises and also resulted in loss of jobs. No doubt, ASF constitutes a major threat to national food security and income generation by the rural poor including women who predominantly own or tend pigs in different parts of the country. In the absence of bovine Rinderpest, ASF ranks highest among transboundary animal diseases that may have serious implication for animal protein supply and availability, for food security for the rural poor, could destabilize socio-cultural life in some areas of the country including stabilization of traditional marriages and burial rites.

Available Technology for Carcass Disposal

These technologies are presented as a hierarchy based on their reliability for pathogen inactivation (OIE, 2003).

Pyre Burning This is an open system of burning carcasses either on-farm or in collective sites fuelled by additional materials of high energy content. This is a well-established procedure that can be conducted on site with no requirement for transportation of the input material. However, this process is contrary to environmental standards for air, water, and soil. It takes an extended period of time and has no verification of pathogen inactivation. In fact, there is a possibility of particulate transmission from incomplete combustion. Further, because the process is open to view, there is a negative reaction and lack of acceptance by the public.

Composting This is a process of aerobic microbiological decomposition conducted in either open or closed systems. It preferably requires prior grinding of tissues and as well the addition of organic material for microbial maintenance. Additionally, mixing or aeration is required to assure homogeneous decomposition. This simple process, which can be conducted on site at low cost, can achieve temperatures of up to 70^{o}C. It does, however, require a significantly extended period of time. Further it is necessary to insure a constant temperature throughout the material for the total time period and it is difficult to verify the effectiveness of pathogen inactivation.

Licensed Commercial Landfill This process involves deposition of carcasses in predetermined and environmentally licensed commercial sites. Because the site has been previously licensed, all environmental impacts such as leachate management, gas management, engineered containment, flooding, and aquifers have already been considered. However, the area is open and uncovered for extended periods, there is a potential emission of aerosols, and there is resistance from the public to such an approach.

Fermentation This process is a closed system of anaerobic microbiological decomposition which requires prior mechanical and thermal treatment and which results in the production of biogas. This process does not inactivate pathogens, but typically uses non-dried rendered product as the input material.

Technologies under Development

Alkaline Hydrolysis Alkaline hydrolysis consists of treating carcasses or tissue in an aqueous alkaline solution at elevated temperatures under pressure. It converts proteins, nucleic acids, and lipids of all cells and tissues into a sterile aqueous solution of small peptides, amino acids, sugars, and soap. What remains are the mineral constituents of the bones and teeth. This process requires specialized equipment and operates at 150^{o} C for three hours. It completely inactivates pathogens with the exception of prions where infectivity is reduced, and is environmentally responsible.

Biosphere Process The biosphere process is a bio-refining technology which employs a biolytic hydrolyzer, operating under high temperature, steam pressure, and internal agitation in a sealed steel vessel. The process produces hydrolysis of protein and carbohydrate materials, fracturing long chain molecules and yielding sterile, high nutrient fertilizer as an output. It operates at 180^{o} C less than 12 atmospheres of pressure for a period of 40 minutes. It inactivates all pathogens and is environmentally sound. Inactivation of prions is still undetermined.

Special Considerations for Prion Diseases

One of the problems in demonstrating the effectiveness of the inactivation of prions (a small protein which is believed capable of infecting cells and causing self to be replicated though it does not contain nucleic acid) is the lack of a simple, rapid and inexpensive test for the presence of the infective agent, especially at low concentrations. The ultimate test is bioassay in a sensitive detector species by an efficient route, but usually this is only relevant in research. Typically this is done using panels of mice bred to be susceptible to particular types of transmissible spongiform encephalopathies (TSEs). However it must be recognized that the mouse to cattle species barrier has been demonstrated to be 500, therefore affecting sensitivity.

Although rendering at 133^{o} C and three bars of pressure for 20 minutes is a defined standard, reductions of infectivity by this technology are in the order of 1:200 - 1:1000. Commercial incinerators have an inactivation rate of one million fold, while burning on pyres has a reduction rate of 90%. (It should be noted that pyres are not suitable for sheep because of the wool and fat.) Alkaline hydrolysis produces a 3-4 log reduction in infectivity over a three hour period. Landfill and deep burial are suggested to have a reduction in infectivity of 98 - 99.8% over three years. Based on this information, rendering, incineration, and alkaline hydrolysis are the most reliable technologies at this time.

The significance of small amounts of infectivity become evident when you consider that experimentally it has been shown that exposure of sensitive species to as little as 1.0, 0.1 or even 0.01 grams of infected nervous tissue can induce infection. Given all of the above, it must be recognized that no process has been demonstrated to be 100% effective in removing TSE infectivity and there will be some residual levels of infectivity remaining after treatment.

Statutory Regulations of Dead Animal Carcass Disposal in Nigeria

Although Onyimonyi *et al.* (2013) reported that Animal Diseases Act of Nigeria provides that where any animal dies of a disease or is slaughtered in accordance with the provisions of this Act or is slaughtered otherwise than in accordance with the provisions of this Act and its carcass is in the opinion of the veterinary officer infected with disease, such carcass shall be disposed-off by burning or in such manner as the veterinary officer may direct. The Act provides for a punishment of 3 months imprisonment or a fine of ₦ 250 ($0.69) for any person who is guilty of

an offence, non-compliance or contravention of this Act. The authors however noted that enforcement of the relevant provision of the statutes mentioned above is practically not in place as no prosecution of any offender of the provisions of these statues is known.

Again, statutory regulations on disposal of dead animal carcass in Nigeria appear not to discuss the disposal of dead animal carcasses where the cause of death is not disease (Onyimonyi *et al*., 2013).These practices no doubt will certainly promote the spread of ASF through movement of the infected pigs, contaminated carcasses and pork products especially during ASF outbreaks. As earlier pointed out, there are apart from disease outbreaks, many situations that could result in death of large number of animals. If we consider the massive destruction and waste of such large scale slaughter, one may thus come to the inevitable conclusion that there must be an alternative which will permit avoidance of this destruction while affecting the required disease control. Therefore the very best method of dealing with disposal of animal carcasses is to avoid the need to slaughter the animals.

Carcass Disposal Methods in Nigeria

The final report of the Avian Influenza Control and Human Pandemic Preparedness and Response Project (2007), identified the following technologies as reliable for carcass disposal/pathogen inactivation: rendering, incineration, compositing, burial, land filling and alkaline hydrolysis [www.jhuccp.org/whatwedo/projects/avian-influenza-control-and-human-pandemic-preparedness-and-response].

On the farm burial, burning and incineration of dead carcass were however observed to be the most practiced methods of disposing dead animals including pigs at major epizootics in Nigeria (Onyimonyi *et al*., 2013; Muhangi *et al*., 2015). Other reported improper disposal of pig carcasses in Nigeria included selling of dead/dying pigs for slaughter, throwing them in lagoons/rivers, bushes, slaughter and sent to market and giving pork from diseased pigs to neighbors (Onyimonyi *et al*., 2013; Obi, 2014; Muhangi *et al*., 2015). Figs 2 and 3.

Fig 2: Dead pigs disposed in garbage collection center

Burying, burning or incinerating are neither done in accordance with the recommendations of OIE (2003) nor follow any international guideline (Figs 2, 3, 4 and 5). According to animal disease emergencies carcass disposal method (www.iowaagriculture.gov), any burial action should be coordinated to ensure the selected site is away from water sources and public lands, has a steep slope greater than 15% and is in suitable soil.

Fig 3: Dead pigs disposed close to a stream

In Nigeria, no vaccine against ASF is presently approved. In the absence of vaccines, the only available option for ASF eradication is stamping out by slaughter and disposal of all infected and potentially infected pigs (FAO, 2001). Thus, all pigs on

infected premises (IPs) and dangerous-contact premises (DCPs), or in a larger area if necessary, must be slaughtered immediately, whether they are obviously diseased or not (FAO, 2017). Owners should be asked to collect and confine their pigs the day before the slaughter team arrives. The animals should be slaughtered by methods that take account of animal welfare and the safety of operatives. The stamping-out approach require technology for animal carcass disposal as an integral component. According to FAO (2001), the carcasses of all pigs that die when there is an incursion of ASF should be disposed safely. This means disposal of the carcasses of animals that have been slaughtered or died naturally of the disease. It must be done in such a way that the carcasses no longer constitute a risk for further spread of the pathogen to other susceptible animals by direct or indirect means, for example by carrion eaters, scavengers or through contamination of food or water.

Fig 4: Decomposing pigs disposed in a bush

This is usually done by deep burial, depending on the nature of the terrain, level of water tables and availability of earth-moving equipment, or by burning, depending on availability of fuels and the danger of starting grass or bush fires (Fig 5).

Fig 5: Dead pigs set for disposal by burning in open air

If in situ disposal is not practical, it may be possible to transport carcasses in sealed vehicles to a disposal point. This should be done within the infected zone. It is not ideal, especially in countries such as in Nigeria where sealed vehicles for such purposes are not available and where vehicles in general are prone to breakdown due mainly to lack of maintenance culture and bad roads. If it must be done, provision should be made for an escort vehicle to disinfect any leakages and initiate salvage operations should the vehicle transporting the pigs develop technical problems or be held up. Whereas there are enabling statutory provisions that clearly stipulates the manner in which dead animal carcass shall be disposed in Nigeria, what is obtainable in practice is totally in contrast with the provisions of the statutes (Onyimonyi *et al*., 2013; Muhangi *et al.*, 2015; Jibril *et al*., 2016).Therefore, the country focuses much on prevention/control measures in the event of ASF outbreak.

Epidemiological Features influencing ASF Control/Eradication Strategies.

A number of epidemiological and other factors that favourably or unfavourably influence the strategies adopted and the ease of control/eradication of ASF in Nigeria have are as follows:

- Among factors that favourably impact on ASF control/eradication strategies include the fact that:
- It is an OIE listed viral disease
- High mortality and morbidity reaching 100 per cent
- It has no vaccine and no cure
- ASF is an emerging transboundary disease
- No domestic livestock species other than pigs is susceptible to ASF;
- Humans are not susceptible;
- *Ornithodoros* ticks that transmit ASF virus have not been described in Nigeria;
- ASF is a highly contagious and clinically apparent disease and disease recognition on the field should therefore be relatively easy.

  Those factors that are unfavourable to easy control/eradication include the facts that:
- The distribution of ASF in West Africa is not static and it is doubtful if some of the neighboring countries have adequate and effective early warning and early reaction capability to enable rapid detection and containment of the disease to the primary focus/i and eventual control or eradication. Therefore the threat of re-introduction of ASF into Nigeria from her neighbors remains high.
- In Nigeria, live pigs and pig meat are important means of spread of the disease. Scavenging and free-roaming pigs often seen feeding on village and abattoir/slaughter slab wastes and garbage play very significant role in spread of the disease among villages in Nigeria in the absence of the sylvatic cycle involving warthogs and *Ornithodorus moubata*.
- ASF virus is resistant to inactivation and may remain viable for long periods in fomites, infected pig tissues, meat and processed pig products;
- Many wild suid species and feral pigs are susceptible to ASF but may not develop overt disease;
- International, inter and intra state trade in live pigs and/or pig meat and products contribute to rapid dissemination of the virus in Nigeria;
- Although ASF is usually clinically apparent, it may be confused with other diseases by an inexperienced animal health personnel;
- Pigs that survive ASF infection may become carriers, although their role in transmitting the virus after about a month is uncertain; their tissues nevertheless remain infective for a period after active shedding has ceased;
- There is no vaccine available for ASF.

Strategies used for ASF Eradication in Nigeria

- In the absence of vaccines, the only available option for ASF eradication is stamping out by slaughter and disposal of all infected and potentially infected pigs. This is a proven method that has succeeded in eradicating ASF and other serious transboundary diseases.
- The main elements of a stamping-out policy for ASF are:
- Zoning of the country into infected zones, surveillance zones and free zones;
- Quarantine procedures to contain the disease, including pig-movement controls and prohibitions of the sale of potentially infected pig products;
- Enhanced epidemiological surveillance for ASF;
- Immediate slaughter of infected and potentially infected in-contact pigs, with prompt and fair compensation to owners;
- Safe burial or burning of carcasses and other infected materials;
- Cleansing and disinfection of infected premises;
- Keeping infected premises/villages without pigs for a safe period.

- Introduction of sentinel pigs for a period of sixty days before restocking.

Conclusion

This report examined methods of carcass disposal in Nigeria during major epizootics such as African Swine fever. Although there is no current reported case(s) of ASF in Nigeria as at the time of this report, our report show that in previous cases of ASF epizootics, the major methods of disposing carcass of dead pigs is by burying, burning or incineration. Other methods like composting, alkaline hydrolysis, licensed commercial landfill, biosphere process, etc are still under development.

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In-House Composting Field Exercise for Broiler Breeders

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June 2018 | Amarillo, Texas

In-House Composting Field Exercise for Broiler Breeders

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Written for presentation at the
6th International Symposium on Animal Mortality Management
Amarillo, TX
June 3-7, 2018

Abstract. *During the US HPAI outbreak of 2015, composting was the main carcass disposal method with 85% of the impacted farms implementing this method to manage their animal carcasses. Even with the successful use of composting at many different types of operations across the country, questions still existed about its applicability for broiler breeder operations. The design and placement of equipment within these operations led many to believe that in-house composting was not practicable on these farms. In fact, in-house composting hasn't been used in the United States at a broiler breeder operation. In-house composting has been used at broiler breeder operations in Canada; however, Canadian broiler breeder housing and equipment designs are more conducive for this practice. Most of the farms impacted by the avian influenza outbreaks in the southeastern United States in 2017 were broiler breeder farms. Due to the challenges of composting within broiler breeder houses and the lack of experience with this method, on-site burial was selected to dispose of the carcasses, feed and manure from these infected farms. To address the questions surrounding the application of in-house composting at broiler breeder operations we collaborated with Virginia's broiler breeder industry to conduct a field exercise. The project proved successful and Virginia's broiler breeder industry intends to utilize in-house composting for future outbreaks of avian influenza.*

Keywords. avian influenza, composting, carcass disposal, broiler breeder

Introduction

The most commonly implemented mass poultry mortality management method during the U.S. 2015 highly pathogenic avian influenza (HPAI) outbreak was composting. The purpose of mass mortality composting was to use biological heating processes to naturally degrade poultry carcasses, inactivate the avian influenza virus, control odors and reduce fly exposure in a safe, biologically, and environmentally sustainable manner.

By definition, composting is a controlled biological decomposition process that converts organic matter into a stable, humus-like product. Composting poultry carcasses is characterized by microbial breakdown of a centralized nitrogen source, the carcasses, which are surrounded by carbon material. The carbon provides energy for microorganisms while the carcass tissues and fluids supply nitrogen for microbial protein synthesis. Typically a base layer (10-15 inches thick; 12-15 feet wide) of sufficiently porous and absorbent carbon material is constructed on the ground. Carcasses, manure and other infected organic material (eggs, feed, etc.) are then placed onto the base layer. The mixture is then capped with 8-12 inches of carbon material. The process begins with an initial breakdown of carcass soft tissue by naturally present microorganisms which produce heat, carbon dioxide, ammonia and volatile organic compounds as by-products. Following soft tissue decomposition, thorough mixing of the carbon material, carcasses and manure promotes a more ideal blend of carbon and nitrogen for optimum composting. Appropriately chosen carbon material traps leachate and odors produced during the process, therefore acting as a biofilter between the carcass and the environment. The continuous high temperatures (> 131°F) achieved through proper composting will destroy most pathogens including the avian influenza virus (Kalbasi et al., 2005). Microorganisms will eventually degrade the carcass leaving only a few remaining bones. Compost that has meet both design and temperature criteria may be approved for release by the appropriate official. This valuable by-product is often land applied as a fertilizer source, recycling nutrients and organic matter to the soil.

Composting mass poultry mortalities is a procedure that can be implemented on most commercial poultry farms. This method requires guidance from a trained composting expert, proper equipment, experienced operators, sufficient carbon, water and an adequate footprint for the compost windrow, either within the poultry house or on the premises. During a disease outbreak, indoor composting is preferred to outdoor composting. When possible, composting inside the poultry house minimizes biosecurity risks and access by scavenging animals. To date, most indoor mortality composting has occurred on commercial turkey farms. Commercial turkey houses are typically built on soil pads. Equipment, such as feeder and drinker lines, can be raised allowing sufficient space for equipment to perform composting procedures. Commercial broiler breeder houses may be built on soil pads, concrete pads or a combination. The houses contain not only feeder and drinker lines, but also manure pits, slats and nest boxes that create

both space constraints and equipment maneuvering challenges. Because of these challenges, questions exist concerning the feasibility of indoor composting on broiler breeder farms.

During the 2017 U.S. HPAI and low pathogenic avian influenza (LPAI) outbreaks commercial broiler breeder operations were largely affected. Due to the perceived challenges and the lack of experience with in-house composting, on-site burial was selected to dispose of the carcasses, feed and manure from these infected farms. To address the questions surrounding the application of in-house composting at broiler breeder operations, a field exercise was conducted in collaboration with Virginia's broiler breeder industry. Both depopulation and in-house composting of a broiler breeder flock were part of the exercise. The objective of the field exercise was to determine the feasibility of in-house composting on a commercial broiler breeder operation.

Methodology

The field exercise was conducted on a commercial broiler breeder operation located in Virginia. The demonstration broiler breeder house was 40 feet by 200 feet in dimension with a 12 foot concrete center scratch area. There were a total of 4,400 hens and roosters in the house at 62 weeks of age (end of production cycle). The birds totaled 38,200 pounds of live weight. Manure and litter were estimated to total 150,000 pounds.

All birds were humanely euthanized via CO_2 gasification under the direction of a licensed veterinarian. Following euthanasia, slats were manually moved from one side of the house to the opposite side. Using skid steers, carcasses and litter were moved from the center scratch area to the manure pit.

Figure 1. Center scratch area following euthanasia.

Figure 2. Cleared scratch area.

Carbon material, consisting of 150 cubic yards of hardwood mulch, was delivered outside the poultry house. Mulch was transported inside to the center scratch area to form an 8 foot wide section of base material. A mixture of carcasses, manure, litter and mulch were then placed on top of the base material. Mulch was used as capping material upon the completion of each section. Additional completed windrow sections were developed moving backward from the egg room to the end doors.

Figure 3. Building compost windrow one section at a time.

Figure 4. Illustration of windrow base, core and cap.

The final windrow dimensions were approximately 11 feet by 200 feet. Only pit manure from one side of the house was used due to space limitations. Pit manure from the opposite side of the house remained undisturbed until turning. Windrow temperatures were monitored daily using long-stem thermometers

Figure 5. Completed in-house windrow.

After 14 days, slats were moved from one side of the house to the opposite side. The compost windrow material was mixed with the remaining undisturbed pit manure and moved outside forming a final windrow. The windrow was capped with mulch and allowed to compost for an additional 14 days. Temperatures were monitored daily.

Figure 6. Completed outdoor windrow.

Results and Discussion

The entire euthanasia process from set-up to completion took 2.5 hours. Manually moving slats from one side of the house to the other side required 50 minutes. Clearing the scratch area of birds and litter, mixing compost material and completing the final in-house windrow took 5 hours. Based on the experience gained during this exercise, process completion times could be drastically shortened.

Average daily windrow temperatures (day 0 to 14) are illustrated in Figure 7.
On day 4, temperatures reached 126°F and then ranged between 125°F and 131°F. Temperatures were slightly lower than the USDA Mortality Composting Protocol for Avian Influenza Infected Flocks target of ≥131°F for 3 consecutive days (Miller et al., 2015). This is most likely due to the large amount of wet, nitrogen-rich manure that was added to the windrow along with the carcasses. With less manure, the carbon to nitrogen ratio would have been more in balance for proper composting. The wet manure may have also contributed to increased anaerobic conditions within the windrow. Temperatures would have likely been higher with less manure addition. It is important to note that these birds were 62 weeks old and at the end of their production cycle. A younger flock would have had less manure to compost.

Figure 7. Average daily windrow temperatures (day 0 to 14).

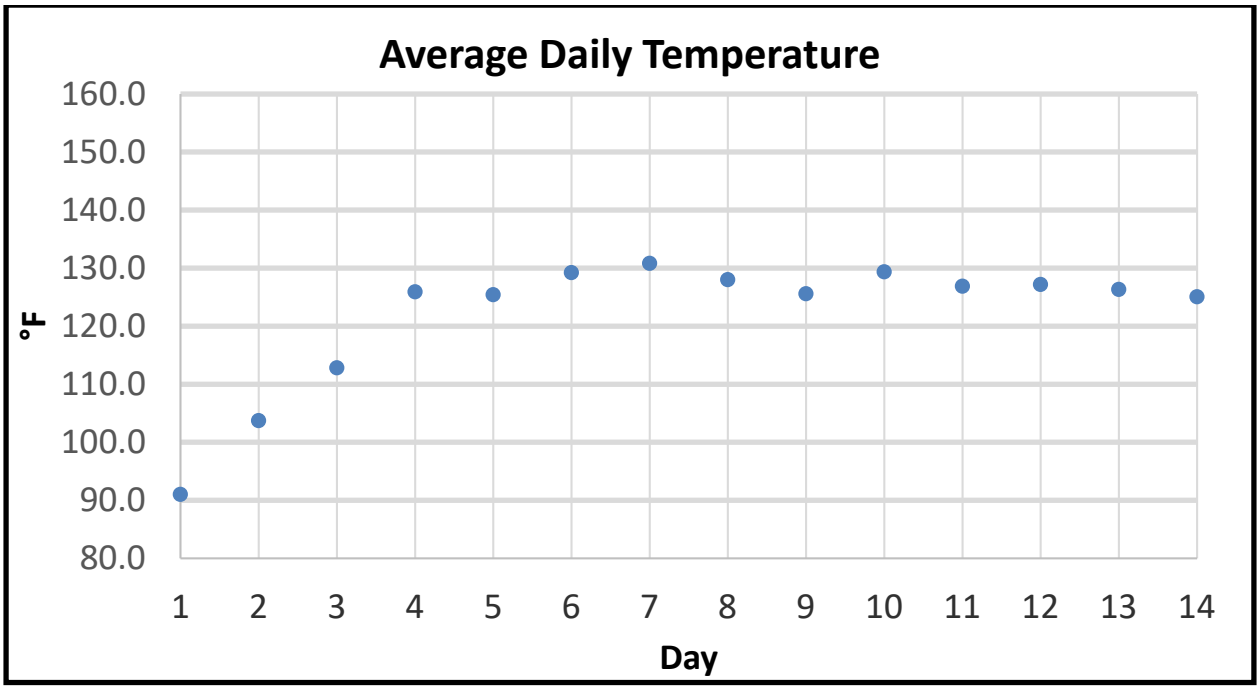
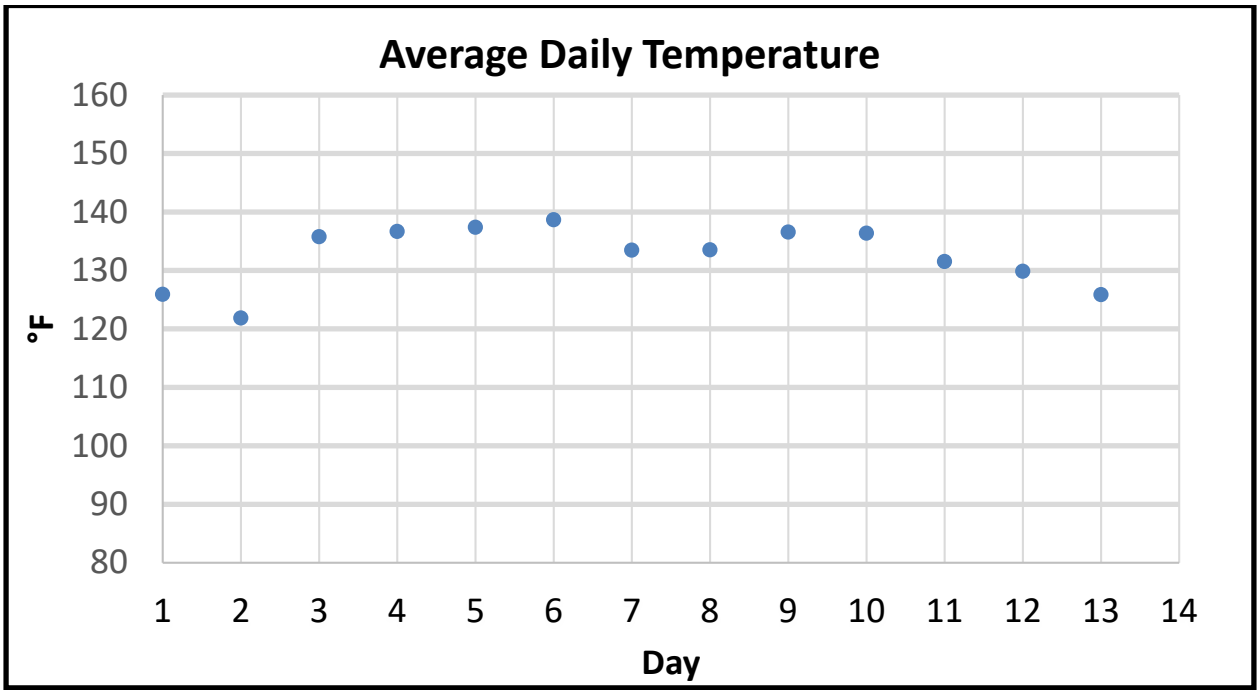


Figure 8 shows average daily windrow temperatures from days 15 to 27. Upon turning and aerating the windrow, temperatures increased to 136°F on day 17 and then ranged from 139°F to 126°F. Temperatures did reach the target of ≥131°F for 3 consecutive days. Mixing and aerating the compost material generally results in an increase in windrow temperatures.

Figure 8. Average daily windrow temperatures (day 15 to 28).



At the 14 day turn, undisturbed pit manure from inside the barn was mixed with the compost windrow material and then moved outside forming a new windrow. In retrospective, a more efficient approach would have been to form a separate outdoor windrow with the remaining manure and fresh carbon material.

Conclusions

The preliminary findings from this field exercise indicate that in-house composting can be successfully implemented on a commercial broiler breeder operation. Suggested modifications to the protocol include leaving more of the pit manure undisturbed until the turn date (day 14). The manure can then be moved outside and composted in a separate windrow with fresh carbon material. This procedure would allow the in-house mortality compost windrow to not become overwhelmed with wet, nitrogen rich manure.

Acknowledgements

Appreciation is extended to Pilgrim's Pride and Allied Industry Partners for their support of this field exercise.

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Avian Influenza Mortality Management Options, Composting and Lessons

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June 2018 | Amarillo, Texas

Avian Influenza Mortality Management Options, Composting Procedures and Lessons Learned

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Written for presentation at the
6th International Symposium on Animal Mortality Management
Amarillo, TX
June 3-7, 2018

Abstract. *The highly pathogenic avian influenza (HPAI) outbreak has become the largest animal health emergency in U.S. history. As of April, 2018, the United States Department of Agriculture (USDA) reports 235 detections (214 commercial facilities and 21 backyard flocks) affecting approximately 50 million birds in 23 states. To date, over $950 million federal dollars have been spent on disease control efforts and indemnities. The infected birds have either died from the disease or been euthanized to control disease spread. Proper carcass management is vital for managing nutrients and controlling disease. Improper disposal may cause odor nuisance, spread disease, and the resulting leachate could negatively impact water sources. Mortality management options that were used during the recent HPAI outbreak include composting, burial, incineration, and landfilling. The most commonly implemented option was mass mortality composting. The purpose of mortality composting during the HPAI outbreak was to use biological heat treatment methods to degrade the carcass, inactivate the avian influenza virus, control odors and reduce fly exposure in a safe, biosecure, and environmentally sustainable manner. As a result of the outbreak, a national composting technical team was formed by the USDA, and a mortality composting protocol for avian influenza infected flocks was published. This presentation will outline mortality management options during an animal disease outbreak and highlight the composting methodology implemented on poultry operations during the HPAI outbreak, as well as the successes, challenges and lessons learned*.

Keywords. avian influenza, composting, carcass disposal

Introduction

The highly pathogenic avian influenza (HPAI) outbreak has become the largest animal health emergency in U.S. history. As of April, 2018, the United States Department of Agriculture (USDA) reports 235 detections (214 commercial facilities and 21 backyard flocks) affecting approximately 50 million birds in 23 states. Impacted farms have remained out of production for several months and trade restrictions have been imposed resulting in economic hardships to both growers and the poultry industry. To date, over $950 million federal dollars have been spent on disease control efforts and indemnities. As a result of the outbreak, a national composting technical team was formed by the USDA, and a mortality composting protocol for avian influenza infected flocks was published (Miller et al., 2015). The last confirmed case of HPAI occurred in March, 2017, and there is concern of future outbreaks due to the continued migration of waterfowl which serve as a reservoir for avian influenza viruses.

Infected birds have either died from the disease or been euthanized to control disease spread. Proper carcass management is vital for both controlling disease and managing nutrients. Improper disposal may cause odor nuisance, spread disease, and the resulting leachate (carcass fluids) could negatively impact water sources. The avian influenza virus may still be present within the carcass, litter, feed or eggs and could be spread by insects, rodents, predators, and subsurface or above ground water movement, as well as through direct contact with other birds, leading to increased disease transmission risks. For these reasons, proper mortality management practices must be implemented immediately following a disease outbreak. Strict biosecurity measures must be adhered to in order to prevent disease transmission from human activity.

Mortality management options that were used during recent avian influenza outbreaks include:

- Composting
- Burial
- Incineration
- Landfilling

The most commonly implemented option was mass mortality composting which will be discussed later.

Burial is a disposal method in many states that may be conducted on-site and quickly if acceptable land mass is available. A site assessment is required to ensure that local environmental guidelines are followed. Common considerations include location, soil type, depth to groundwater, and distance to waterways. Sandy soils, karst topography or areas with a high water table pose a risk of contaminating groundwater supplies. Researchers have demonstrated the potential transport of carcass leachate components, such as nutrients and bacteria, from burial pits to groundwater (Ritter and Chirnside, 1995; Myers et al., 1999; Glanville, 2000; Pratt and Fonstad, 2009). Avian

influenza has been reported to survive for weeks in water depending on variables such as temperature, salinity and pH (Brown et al., 2009) and has survived more than one year in manure-amended soil (Elving et al., 2012). Furthermore, portions of buried carcass can persist for years in an anaerobic environment. During construction projects on former poultry farms, old burial pits have been discovered that contain intact birds (B. Malone, personal communication, August, 21, 2015). For these reasons, burial should be given careful consideration when implementing this method of carcass disposal.

Proper incineration requires a closed air unit, can be conducted on-site and is a pathogen inactivation procedure. Depending on the state, an air quality permit may be required. Check with state officials. Several incinerators are required during a large animal disease outbreak. Fuel costs and carcass throughput are important factors to consider especially when managing large amounts of carcass material. For large poultry operations, incineration may be adopted in combination with other mass mortality management practices.

If locally available, poultry mortalities may be disposed of at a licensed landfill that accepts animal carcasses. Permitted landfills are an important option during a poultry disease outbreak. These landfills must have necessary environmental controls in place to manage carcasses. Landfilling is considered a form of burial; however, permitted landfills are designed to contain leachate and allow for gas management, which protects the environment, unlike unlined burial. Landfilling can be convenient and fast for mass mortality disposal once approved by the appropriate regulatory agency and the landfill managers. Considerations include tipping fees, additional handling of mortalities and transportation of infected carcasses. Strict biosecurity measures must be followed during transportation and disposal. Proper packaging of carcasses in sealed roll-off containers is required, along with proper cleaning and disinfection procedures to minimize biosecurity risks.

Mass mortality composting was implemented on the majority of infected poultry operations during avian influenza outbreaks. The purpose of mass mortality composting was to use biological heating processes to naturally degrade poultry carcasses, inactivate the avian influenza virus, control odors and reduce fly exposure in a safe, biologically, and environmentally sustainable manner.

By definition, composting is a controlled biological decomposition process that converts organic matter into a stable, humus-like product. Composting poultry carcasses is characterized by microbial breakdown of a centralized nitrogen source, the carcasses, which are surrounded by carbon material. The carbon provides energy for microorganisms while the carcass tissues and fluids supply nitrogen for microbial protein synthesis. Typically a base layer (10-15 inches thick; 12-15 feet wide) of sufficiently porous and absorbent carbon material is constructed on the ground. Carcasses, manure and other infected organic material (eggs, feed, etc.) are then placed onto the base layer. The mixture is then capped with 8-12 inches of carbon material. The process begins with an initial breakdown of carcass soft tissue by naturally present microorganisms which produce heat, carbon dioxide, ammonia and

volatile organic compounds as by-products (Berge et al., 2009). Following soft tissue decomposition, thorough mixing of the carbon material, carcasses and manure promotes a more ideal blend of carbon and nitrogen for optimum composting. Appropriately chosen carbon material traps leachate and odors produced during the process, therefore acting as a biofilter between the carcass and the environment. The continuous high temperatures (> 131°F) achieved through proper composting will destroy most pathogens (Kalbasi et al., 2005; Kalbasi et al., 2006; Wilkinson, 2007) including the avian influenza virus (Elving et al., 2012). Microorganisms will eventually degrade the carcass leaving only a few remaining bones. Compost that has meet both design and temperature criteria may be approved for release by the appropriate official. This valuable by-product is often land applied as a fertilizer source, recycling nutrients and organic matter to the soil.

Composting mass poultry mortalities is a procedure that can be implemented on most commercial poultry farms. This method requires guidance from a trained composting expert, proper equipment, experienced operators, and sufficient carbon, water and open space. During a disease outbreak, indoor composting is preferred to outdoor composting. When possible, composting inside the poultry house minimizes biosecurity risks and access by scavenging animals. Since carcasses are contained on-farm, composting can be more biosecure compared to methods that transport carcasses off-farm. The USDA avian influenza mortality composting protocol requires a 28 day composting process. Hence, in-house poultry mortality composting may delay poultry house cleaning and disinfection efforts resulting in extended down times as compared to other disposal methods. However, options may exist to move compost windrows outdoors following a 14 day compost process. Finally, proper composting can degrade poultry carcasses into a useful soil amendment and fertilizer.

Figure 1. Poultry carcasses being buried.

Figure 2. Incineration of poultry carcasses.

Figure 3. Landfill disposal of poultry.

Figure 4. Indoor turkey mortality compost windrow.

Conclusions

Each mortality management practice has pros and cons and should be carefully considered, based on the situation at hand. Site assessment, biosecurity, severity of outbreak and available resources are key elements that influence the decision-making process when selecting the most appropriate option. Regardless of the method chosen, all poultry farming operations should have a detailed catastrophic mortality management plan in place should a disease outbreak occur. Necessary supplies, labor and equipment should be outlined. The plan should be developed with assistance from the respective poultry company and state regulatory agency. Finally, time is critical when responding to any disease outbreak. To reduce odor and exposure to flies and scavengers, a mortality management practice should be implemented immediately and completed rapidly, yet efficiently.

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Federal 3D Priorities Update

Lori P. Miller, USDA APHIS

June 2018 | Amarillo, Texas

USDA
United States Department of Agriculture

FEDERAL 3D PRIORITIES UPDATE

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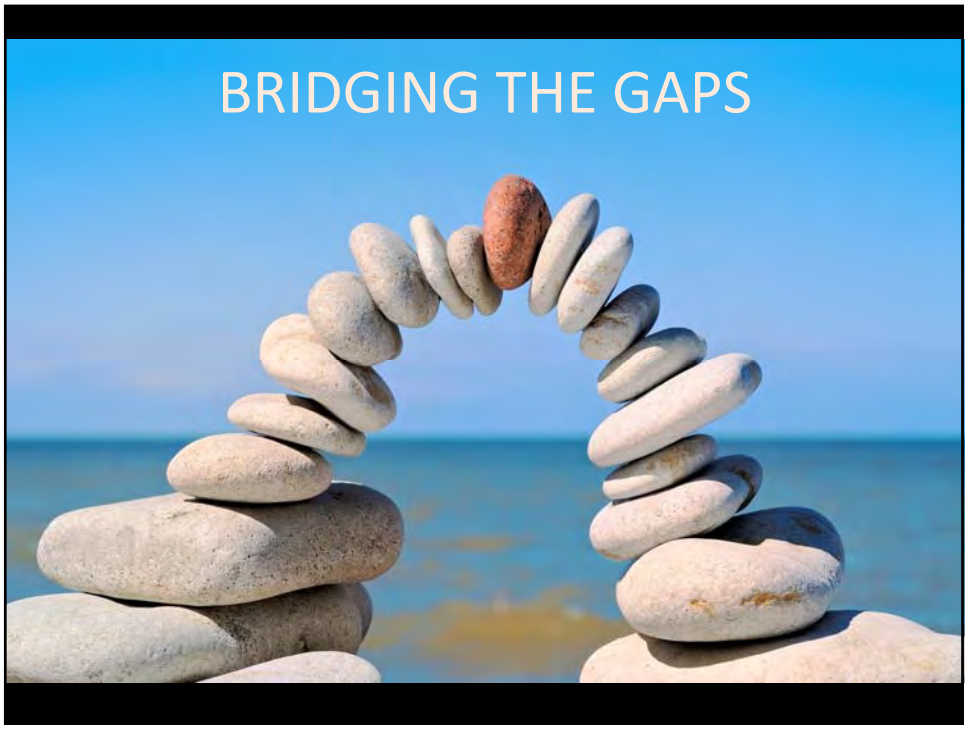
JUNE, 2018



LARGE ANIMAL COMPOSTING PROTOCOL

Developed by APHIS-Led Composting Technical Team; available on APHIS website

RENDERING WORKSHOP – July 2017
Identifying obstacles to emergency rendering and discussing options to overcome them

MOBILE INCINERATION
2017 Iowa field test was effective with low air emissions

WASTE TO ENERGY

Indiana exercise successfully demonstrated feasibility for spent laying hens

SUSTAINABLE CARCASS MANAGEMENT COLLABORATION WITH FAO

NON-FREEZING WASH TUNNEL AND ROBOTS

Autonomous robot climbs into livestock carrier to clean and disinfect interior

CARCASS MANAGEMENT EXPOSURE ASSESSMENT

FMD Scenario Sensitivity Analysis

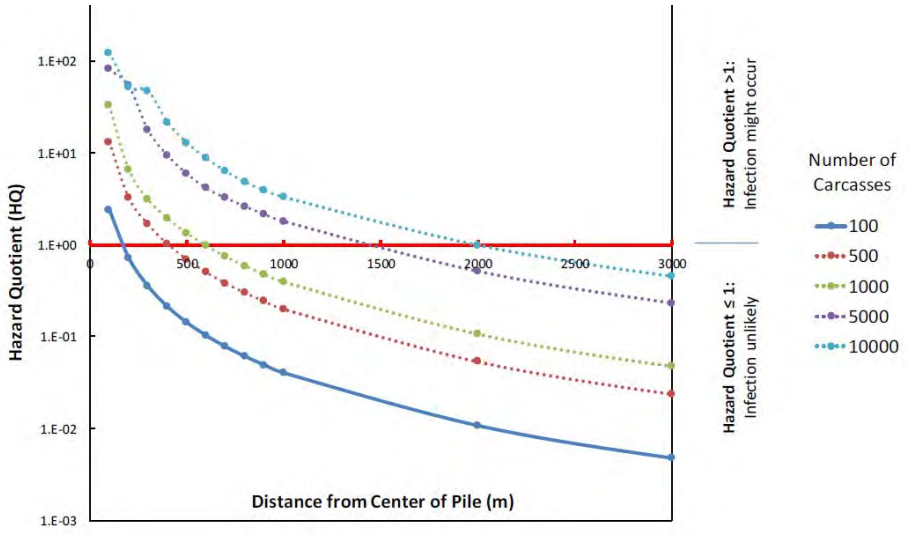


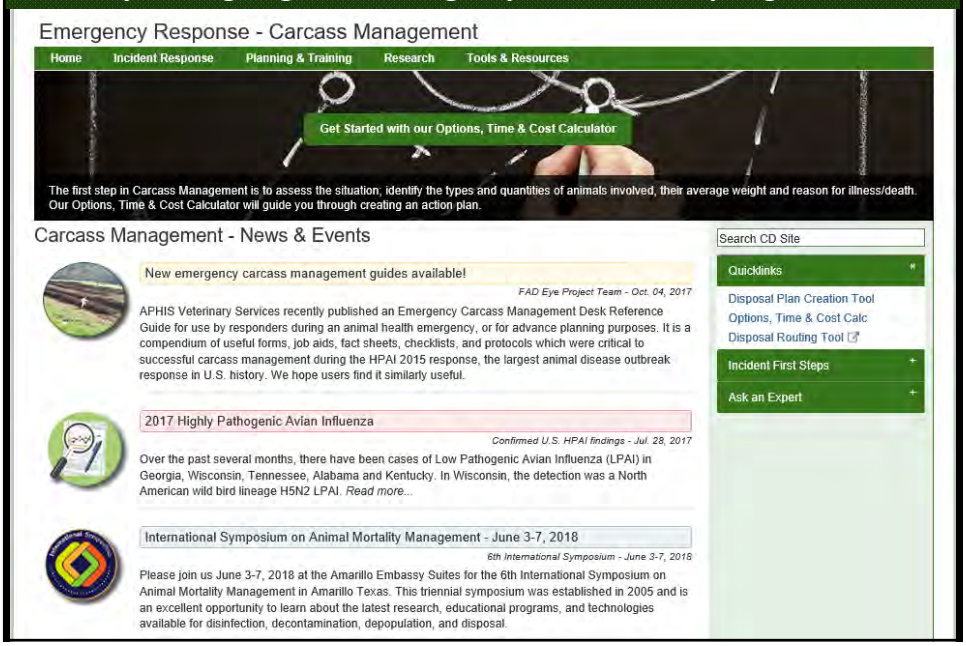
Figure 4.3. Sensitivity Analysis for the Number of Carcasses, Inhalation HQs for Dairy Cattle with Distance from the Storage Pile.

LEACHATE MANAGEMENT EXPOSURE ASSESSMENT

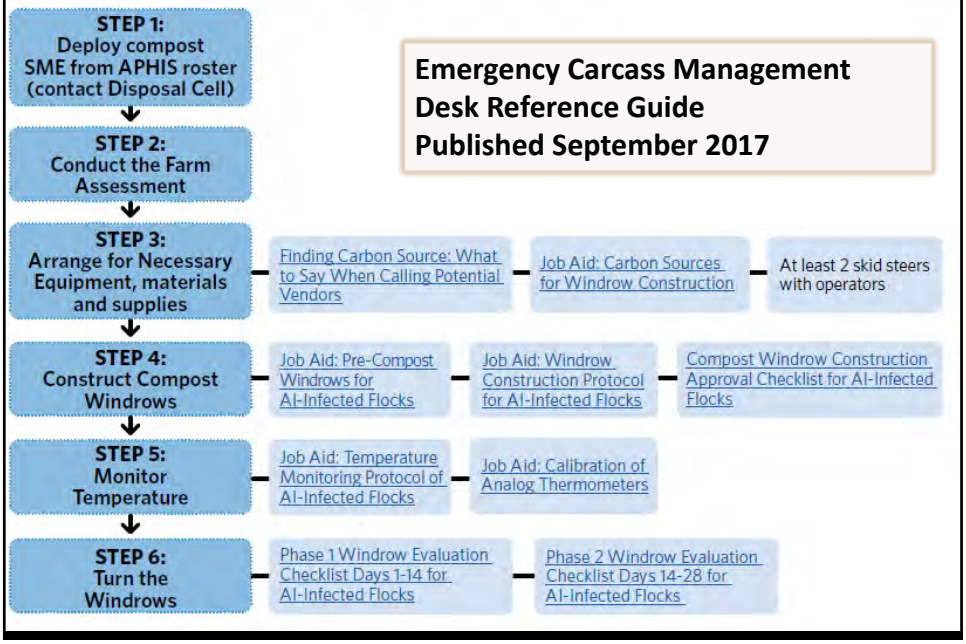
Findings: landfills concerned that viruses survive in landfill leachate; assessment finds low risk under worst-case scenario

CARCASS MANAGEMENT DASHBOARD AND TOOLS

Currently undergoing beta testing; expected online Spring ’18.



Overview of In-House Composting Process



USDA United States Department of Agriculture

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Case Study of Enteric Illness in Responder Associated with 2015 HPAI Carcass Disposal Response

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June 2018 | Amarillo, Texas

Case Study of Enteric Illness in Responder associated with 2015 HPAI Carcass Disposal Response

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Written for presentation at the
6th International Symposium on Animal Mortality Management
Amarillo, Texas
Embassy Suites Hotel and Conference Center

Abstract. *A composting Subject Matter Expert (SME) was assigned to work with an HPAI positive turkey flock for composting of turkey carcasses and contaminated bedding, feed and other materials. The SME developed enteric illness after working with responders to construct compost windrows. The SME was wearing the recommended PPE for the expected response activities. Exposure to an enteric pathogen may have occurred by skin contact with contaminated fluids. The related*

presentation will review the PPE used by the patient during the response, possible exposure routes, the current APHIS VS PPE guidance document and possible recommendations for prevention of exposure during future animal disease responses. The presentation will also include an overview of Campylobacter, Salmonella, SARS and Ebola Virus associated with occupational exposure.

Keywords. Highly Pathogenic Avian Influenza (HPAI), composting, Personal Protective Equipment (PPE), donning PPE, doffing PPE, Enteric illness*, Campylobacter*, *Salmonella*

Case History:

The case patient was a composting Subject Matter Expert (SME) responding to a Highly Pathogenic Avian Influenza (HPAI) positive turkey grow out premises. The patient was working in the poultry houses providing technical information during construction of composting windrows for turkey carcass management. The patient was wearing Tyvek coveralls with hood, hairnet, nitrile gloves (two pairs taped with duct tape at wrist to coveralls), a fitted half face respirator, rubber boots, boot covers (two pairs taped at top of boot covers with duct tape to coveralls), and goggles. The PPE followed the FY2016 HPAI Response Interim Recommendations for expected exposure (See FY2016 HPAI Response, Interim Recommendations on PPE for Selected Activities, April 25, 2016) [1]

The case patient reported a splash to the exposed skin on their face with tissue from turkey carcasses during movement to construct windrows. The patient was unable to rapidly take off the PPE to clean contaminated skin because their gloves were covered with fluid from handling the turkey carcasses. Assistance for responders while donning (putting on) and doffing (taking off) PPE was dependent on staffing and not always available.

Hand sanitizer was provided during doffing of PPE, however running water for hand washing was not usually available. Exposure to an enteric pathogen may have occurred by contamination of hands, eyes or mucous membranes during doffing of PPE or sweating which could spread contamination to eyes or mucous membranes.

The case patient reported clinical signs of abdominal cramping, severe diarrhea, blood in stool, nausea, and weakness that occurred after an incubation period of approximately 12 hours. The patient left the response to seek medical care and was treated symptomatically with antibiotics and supportive care for dehydration and enteritis. The physician's diagnosis was probable *Campylobacter* enteritis based on poultry exposure history and clinical signs. Diagnostic testing for *Campylobacter* was not available at the time of the medical examination and treatment.

The case patient recovered after approximately 10 days and was able to resume HPAI composting response activities.

Discussion:

Campylobacter is a gram negative bacteria in the genus *Campylobacteriacae, Campylobacter jejuni* is the species most commonly identified in human infections. Exposure is often associated with eating undercooked poultry or foods contaminated by raw poultry. The incubation period is usually 2-4 days and clinical signs include fever, diarrhea, abdominal pain, nausea and vomiting. In some cases more severe disease such as septicemia, Guillain-Barre syndrome, irritable bowel syndrome

or arthritis may develop. Diagnosis is confirmed by isolation of *Campylobacter spp*. from a clinical specimen and a probable case by detection of *Campylobacter spp*. by PCR. [2]

Salmonella spp. are gram negative facultative anaerobic rod bacteria in the family Enterobacteriaceae. They are classified into over 2500 known serovars or serotypes. *Salmonella* serotype Typhimurium and *Salmonella* serotype Enteridis are the serotypes that most often cause human disease. Salmonella is the most common cause of foodborne illness worldwide. Exposure can be associated with eating incompletely cooked eggs, poultry, other foods contaminated with the bacteria and contact with live poultry, reptiles, etc. In 2012, USDA-FSIS conducted a nationwide Microbiological survey of raw chicken parts and found an estimated 24% prevalence of *Salmonella* and 21.4% *Campylobacter* contamination. The incubation period for *Salmonella* infection is usually 12-72 hours. *Salmonella* infection causes diarrhea, fever, abdominal cramps and in rare cases septicemia. Clinical signs last 4-7 days and patients usually recover without treatment. [3,4,5]

From 2008-2011, 29 workers at a poultry processing plant were diagnosed with *Campylobacter* infection. A plant health hazard evaluation was completed and the majority of cases occurred in employees working in the live hang area. The plant instituted engineering controls including improving ventilation, sanitation and training in English and Spanish related to hand hygiene and use of PPE (specific plant worker PPE was not provided in article). [6]

During the 2013-2015 Ebola Virus outbreak response in West Africa, more than 23,000 cases of Ebola were diagnosed. Over 880 of the Ebola cases were in health care workers and of these 512 died. There were shortages of PPE and difficult working conditions that contributed to exposure of health care workers. Many of the medical responders who became infected with Ebola were using PPE recommended by Medecins Sans Frontieres (MSF) including coverall or gown, hood to cover the head, mask covering nose and mouth (N95), goggles, double layer of gloves, rubber boots, and plastic apron. Studies of routes of Ebola virus exposure in health care workers showed that one of the main exposure routes was accidental exposure during doffing of PPE. CDC and other response agencies implemented changes in PPE and infection control including assignment of a trained observer to supervise each step of donning and doffing PPE, providing an assistant for responder during donning and doffing, designating a separate area for donning and doffing PPE, disinfecting gloves and contaminated surfaces during doffing and other control measures. [7,8,9]

During the 2003 SARS outbreak in Hong Kong, 25% of the SARS cases occurred in healthcare workers. After March 2003, infection control measures for treatment of SARS patients were mandatory and included training on transmission of SARS and the use of N95 mask, cap, gown, gloves, and goggles. However health care workers continued to become infected. Lau et al conducted a case control study to determine risk factors for transmission of SARS to health care workers. Significant risk factors associated with SARS infection were perceived shortage of PPE, less than 2 hours of infection control and PPE training, and inconsistent use of PPE.[10]

In a study by Tomas et al of the frequency and locations of the contamination of skin and clothing of health care workers during PPE doffing using fluorescent lotion as a marker, the author found that contamination occurred in 46% of doffing simulations. After training and practice in PPE doffing and use of fluorescent lotion for visual feedback, the contamination during glove and gown removal decreased to 18.9%. The most common sites of contamination were palms of hands, wrists and fingers during removal of contaminated gloves and neck, chest, and hands during removal of contaminated gowns. [11]

Possible future preventive steps to limit exposure of responders to pathogens:

1) Utilize Face shields along with goggles and N95 mask or whole face respirators during high risk activities such as movement of animal carcasses, construction of windrows, and turning of windrows where responder could be exposed to splashing or aerosolization of contaminated liquids. If splashing or exposure to fluids may occur during carcass disposal, avoid the use of N95 masks that can become ineffective for filtration of particulates when wet.

2) Ensure the composting SME's role is only to provide technical information during compost windrow construction, windrow temperature monitoring and turning and not actively assisting with handling carcasses and moving composting materials.

3) During carcass disposal activities, provide responders assistance with donning and doffing PPE by assigning a dedicated safety officer or personnel trained in the use of PPE. Safety personnel can also assist responders with onsite guidance and training on PPE use, PPE supplies, communications, and assistance with PPE during water/rest breaks to prevent exposure of responders to pathogens. It is important that those assisting are also wearing the appropriate PPE.

4) Increase training and practice sessions for donning and doffing PPE. Recommend periodic outreach and practice sessions in District and area offices through webinars, hands on training and exercises by safety and health personnel, safety officers and field staff who routinely use PPE. Include the use of fluorescent lotion as a marker for contamination during training and practice sessions.

5) When possible during disease outbreak response, provide running water and soap for hand washing and cleaning skin that may have been contaminated during the response activities and doffing of PPE. If running water is not available provide disinfecting wipes and hand sanitizer.

Conclusions

During an animal disease outbreak, responders can be exposed to endemic zoonotic diseases such as *Campylobacter*, and *Salmonella* as well as foreign animal diseases such as HPAI. Providing regular PPE training and exercises, assistance with donning and doffing PPE during responses, and regular review of PPE guidance may decrease the risk of responder exposure and possible spread of pathogens to other animal premises.

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CASE STUDY OF ENTERIC ILLNESS IN RESPONDER ASSOCIATED WITH 2015 HPAI CARCASS DISPOSAL RESPONSE

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MAY 10, 2018
SPEAKER: LORI MILLER, PE, SENIOR STAFF OFFICER/ENVIRONMENTAL ENGINEER

Introduction

- Composting SME working with responders on HPAI positive turkey grow out premise carcass disposal developed enteric illness
- PPE used in response may have been inadequate to protect from possible exposure routes
- Other responses involving PPE use have resulted in Occupational exposure to zoonotic pathogens
- Recommend reviewing and updating PPE guidance

USDA United States Department of Agriculture

Case Patient History

- Case patient, composting SME on HPAI positive turkey grow out premise
- Providing technical input for windrow construction for disposal of turkey carcasses and contaminated bedding/feed
- Case patient PPE: Tyvek coveralls with hood, hairnet, nitrile gloves, fitted half face respirator, rubber boots, boot covers and goggles
- Exposed skin on face splashed with infected tissue

3

USDA United States Department of Agriculture

Case Patient History

- Exposure may have resulted from possible contamination of hands, eyes, or mucous membranes during doffing of PPE or sweating which could spread contamination to eyes or other mucous membranes
- Patient clinical signs after approx. 12 hours incubation period:
  - abdominal cramping
  - severe diarrhea
  - blood in stool
  - nausea and weakness

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USDA United States Department of Agriculture

Case Patient History

- Patient treated by physician supportive care & antibiotics for dehydration and enteritis
- Tentative diagnosis *Campylobacter* enteritis
  - Diagnostic testing for Campylobacter not available
- Recovery after 10 days and returned to HPAI composting response

5

USDA United States Department of Agriculture

Discussion

- Responders to animal disease outbreaks can be exposed to zoonotic pathogens
- Possible routes of exposure to animal disease responders may be similar to plant workers and health care workers
- Preventing exposure of responders to pathogens includes
  - Engineering controls/Infection control program
  - Training/Administrative Controls
  - PPE

6

USDA United States Department of Agriculture

Discussion-Enteric Pathogens

- Enteric pathogens
  - *Campylobacter*
    - gram negative bacteria in the genus *Campylobacteriacae*
    - In 2012 FSIS Microbiological baseline data study for raw chicken parts, 21.4% positive for *Campylobacter*
  - *Salmonella*
    - Gram negative bacteria in genus Enterobacteriaceae
    - In 2012 FSIS study, 24% raw poultry parts positive for *Salmonella* spp.
    - Most common cause of foodborne disease

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USDA United States Department of Agriculture

Discussion-*Campylobacter*

- Perio et al, Occupational exposure to *Campylobacter* in Poultry processing plant
- 29 cases of *Campylobacter* reported in workers 2008-2011 primarily in live hang area
- Plant health hazard evaluation
- Plant implemented engineering controls including improved ventilation, sanitation and training in English and Spanish related to hand hygiene and PPE

8

USDA United States Department of Agriculture

Discussion-Ebola Virus

- 2013-2015 Ebola outbreak West Africa 23,000 cases with 880 health care workers infected
- Health care workers used Medecins Sans Frontieres (MSF)/CDC PPE: coverall/gown, hood, N95 mask, goggles, double layer gloves, rubber boots, plastic apron
- Some health care workers wearing recommended PPE still exposed and developed Ebola virus infection
- Contamination of skin or mucous membranes during doffing PPE determined to be one route of exposure

9

USDA United States Department of Agriculture

Discussion-Ebola Virus

- CDC implemented infection control/engineering controls to prevent exposures including
  - Observer to monitor that all steps of donning and doffing PPE completed correctly
  - Assistant for responder during donning and doffing
  - Designate separate area for donning PPE and doffing PPE
  - Disinfection of gloves and contaminated surfaces during doffing and other control measures

10

USDA United States Department of Agriculture

Discussion-SARS

- 2003 SARS outbreak in Hong Kong, 25% of cases occurred in Health care workers
- Mandatory infection control measures in place
  - Training on infection control, PPE, SARS virus transmission
  - PPE: N95 mask, cap, gown, gloves, goggles
- Lau et al case control study to determine risk factors for continued transmission of SARS to health care workers
- Significant risk factors for SARS infection in health care workers
  - Perceived shortage of PPE
  - Less than 2 hours of infection control and PPE training
  - Inconsistent use of PPE

11

USDA United States Department of Agriculture

Discussion-Doffing PPE

- Tomas et al studied frequency and location of contamination on skin and clothing during PPE doffing using fluorescent lotion marker
- Contamination occurred in 46% of doffing simulations in health care workers
- Additional training and practice doffing PPE and using fluorescent lotion for visualization of contamination, contamination decreased to 18.9%
- Most common sites of contamination for contaminated gloves: palms of hands, wrists and fingers
- Most common sites of contamination for contaminated gowns: neck, chest and hands

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USDA United States Department of Agriculture

Discussion-Prevention

- Utilize Face shields with goggles and N95 mask or whole face respirators during high risk activities such as movement of animal carcasses, construction of windrows, and turning of windrows where responder could be exposed to splashing. Avoid use of N95 mask if mask can become wet from splashing.
- Clarify that composting SME's role is to provide technical information during compost windrow construction, windrow temperature monitoring and turning and not actively assist with handling carcasses and moving composting materials.

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USDA United States Department of Agriculture

Discussion-Prevention

- Provide responders safety assistant for donning and doffing PPE, water breaks, communication. Safety assistants also wear appropriate PPE.
- Recommend periodic outreach, training and practice sessions for donning and doffing PPE. Use fluorescent lotion during practice.
- Provide running water and soap for hand washing and cleaning skin during the response activities and doffing of PPE. If running water is not available provide disinfecting wipes and hand sanitizer.

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USDA United States Department of Agriculture

Conclusions

- Responders may be exposed to endemic and foreign animal zoonotic diseases during disease outbreaks
- Occupational exposure
  - HPAI disease outbreak response: *Campylobacter*
  - Poultry processing plant: *Campylobacter*
  - Medical Workers: Ebola and SARS
- Limit exposure of responders
  - engineering controls/infection control program
  - PPE training and exercises
  - Assistance donning and doffing PPE

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USDA United States Department of Agriculture

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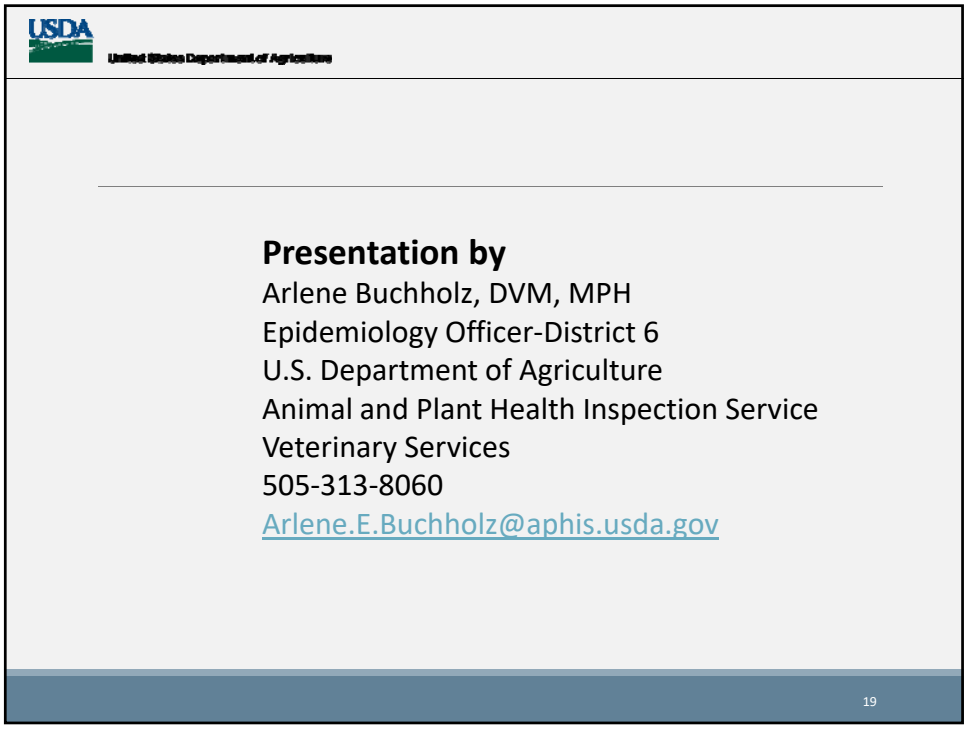
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Filth Fly Activity Associated with Composted and Non-Composted Beef Cadavers and Lab Studies on Volatile Organic Compounds

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June 2018 | Amarillo, Texas

Filth Fly Activity Associated with Composted and non-composted Beef Cadavers and Laboratory Studies on Volatile Organic Compounds

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Written for presentation at the
6th International Symposium on Animal Mortality Management
Amarillo, Texas
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Abstract. *Commercial livestock facilities are faced with the challenge of managing large amounts of waste including manure and animal mortalities. One method of disposing of dead animals is composting. The cadavers are enveloped in carbon material that creates a barrier between the dead tissue and the surrounding environment. Dead tissue can release materials that not only contaminate the soil but also the groundwater and nearby surface water. Animal cadaver composting is designed to facilitate decomposition without the aid of carrion feeding insects and reduce the presence of common pathogens associated with animal waste and dead tissue. The aim of this study was to evaluate insect activity associated with composted and exposed beef cadavers, specifically filth flies that can serve as mechanical vectors of important human pathogens such as E. coli 0157:H7. Greater numbers of all types of arthropods were trapped overall at the exposed animal site than the composted animal site. Most importantly, the number of filth flies was significantly lower at the composted site (P=0.0009). Laboratory analysis of volatile organic compounds from composted and non-composted rats indicated that known fly attractants such as dimethyl disulfide may be inhibited by the composting process. Implementing composting programs at livestock facilities could reduce the risk of flies spreading harmful pathogens to surrounding areas.*

Keywords. bovine cadaver, decomposition, carcass disposal, filth flies

Introduction

Catastrophic events such as disease outbreaks and natural disasters can dramatically increase the number of livestock mortalities, and these circumstances require a safe and effective means of carcass disposal. Exposed livestock cadavers can contain harmful zoonotic pathogens that can be transmitted to other livestock animals (Lloyd-Smith et al. 2009), and many filth flies are competent mechanical vectors of pathogenic microorganisms that can be acquired from animal manure and animal carcasses (Greenberg 1973, Graczyk 2001). Filth flies have also been implicated in the transmission of antibiotic resistant bacteria (Zurek and Ghosh 2014). Filth flies are potential vectors for contaminating produce on-farm, and therefore their control is an essential component of food safety (Talley et al. 2009, Wasala et al. 2010). Conditions within a compost pile reach temperature that theoretically should be too high for filth fly development, and the envelope of composting materials should also prevent flies from accessing the carcass. Composting cadavers could reduce filth fly activity overall and lessen the public health risks associated with the disposal of livestock mortalities. The aim of this study was to evaluate the activity of carrion feeding insects, specifically filth flies, associated with composted and exposed bovine cadavers, and to determine what effect, in any, the composting process has on the emission of volatile organic compounds that play in important role in filth fly olfaction and carrion detection.

Materials and Methods

Carcass sites: Bovine carcasses, Bos taurus, were acquired from the Oklahoma State University's Willard Sparks Beef Research Center and North Lake Carl Blackwell Beef Research Range in Stillwater, OK. Each site was in a partially wooded habitat with similar surrounding vegetation. Both field sites were equipped with a 3m x 3m fence, approximately 1.5 m high, to keep large scavenger animals from disturbing the carcasses and compost. All animals were obtained within 12 hours of death, and each set of animals, one composted and one exposed for each replicate, was observed simultaneously throughout the entire period of decomposition.

Temperatures inside of the compost piles and under the exposed cadavers were monitored throughout the period of decomposition. A HOBO U23 Pro v2 External temperature/relative humidity data logger with a sensor on the end of a 2 m long cable was placed directly under the posterior end of each carcass (U23-002, Onset Computer Corp. Bourne, MA). A mixture of different types of hard and soft wood chips, associated leaf litter, and sawdust obtained from the OSU Botanical Garden was used for the composting media, and the material was moistened with water per the standard recommendations for mortality compost which is approximately 50% moisture (Payne and Pugh 2010). Following standard recommendations for C:N ratio of 25:1, composted animal sites were constructed by establishing a pad of composting media that was approximately 0.5 m thick with an envelope of approximately 0.5 m surrounding the carcass to ensure adequate insulation (Rynk 1992, Payne and Pugh 2010). Before burial in composting media, the rumen of each animal was punctured in order to avoid excessive bloating. The exposed and composted cadavers remained undisturbed throughout the entire period of decomposition.

Insect Activity: Malaise traps were installed to trap flying insects visiting each carcass site. The traps were fabricated specifically to be suspended above each carcass or compost pile without disturbing the sites with poles or stakes. Each cadaver was also examined for eggs and larvae

upon arrival, and additional activity outside of malaise traps, if any, was noted. Samples were collected every 2 to 4 days and frozen for subsequent identification. They were collected from both sites on the same days, and sampling continued throughout decomposition until visual inspection of the exposed carcass determined that it has progressed to the skeletal stage where only bone and hair remained (Lord and Goff, 2003).

Analysis of Volatile Organic Compounds: Volatile organic compounds (VOCs) were sampled using 75 µm carboxen®/polydimethylsiloxane solid-phase microextraction (SPME) fibers (Supelco #57344-U, Sigma-Aldrich Co. LLC, Bellfonte, PA). Individual compost containers were fitted with a large plastic jar to catch any VOCs emitted over a period of 48 hours, and Parafilm® (Bemis Company Inc., Neenah, WI) was used to completely seal the edges around each lid. SPME sampling was done on days 1, 5, 6, 12, 41, and 48 for all rats, and these dates were chosen based on the stage of decomposition observed in the exposed carcasses. The headspace in each container was homogenized by mild agitation, and individual fibers were exposed in the headspace for a period of 20 minutes (Hoffman et al., 2009). The plastic jars and lids were removed after each sampling event in order to release remaining odorant compounds and purify the headspace between samples. The samples were immediately analyzed using gas chromatography/mass spectrometry (GC/MS).

The instrument used was an HP6890 gas chromatograph (GC) with a 5973 mass selective detector (MSD) (Agilent Technologies, Palo Alto, CA). The instrument was equipped with a DB5-MS capillary column 30 meters long with an internal diameter of 0.25 mm, a film thickness of 0.25 µm, and a SPME injection port liner operated at 250°C. The carrier gas used was helium and the flow rate was set at 1.5mL/minute. The oven temperature was set at 40°C to begin, held for 1 minute, ramped to 80°C at 3°C/minute, then up to 120°C at 10°C/minute, and lastly raised to 260°C at 40°C/minute. The total run time for the program was 21.83 minutes. The MSD was scanned 10 to 700 amu at a rate of 2.94 scans per second. Protocols for this study were adapted and modified from Hoffman et al. (2009). Data was collected using ChemStation and the spectra deconvoluted using AMDIS32 software. Compounds were identified using the NIST mass spectral library (National Institute of Standards and Technology, Gaithersburg, MD).

Statistical Analysis: Data from malaise trap samples collected throughout the entire period were pooled for each treatment. A two-sample t-test was conducted to determine any difference between the mean total abundance of arthropods and the mean total abundance of filth flies (SAS. Version 9.3; SAS Institute, 2013).

Results and Discussion

The mean total abundance of all insects collected from malaise traps throughout both replicates was numerically higher at the exposed carcass site, not statistically significant from the composted site ($F=1.05$, $df=1$, $p=0.2730$). The mean number of filth flies trapped at the exposed cadaver site was significantly higher than those trapped at the composted site ($F=1.96$, $df=1$, $p=0.0009$) (Fig. 1). Temperatures within the compost pile were consistently much higher than both the ambient temperature and temperature under the exposed carcass for both replicates reaching their peak at 48°C and 59°C for the first and second round, respectively. During the

initial heat cycle, which corresponded with the active decay stage in the exposed carcasses, odor was detectable only in close proximity to the compost pile.

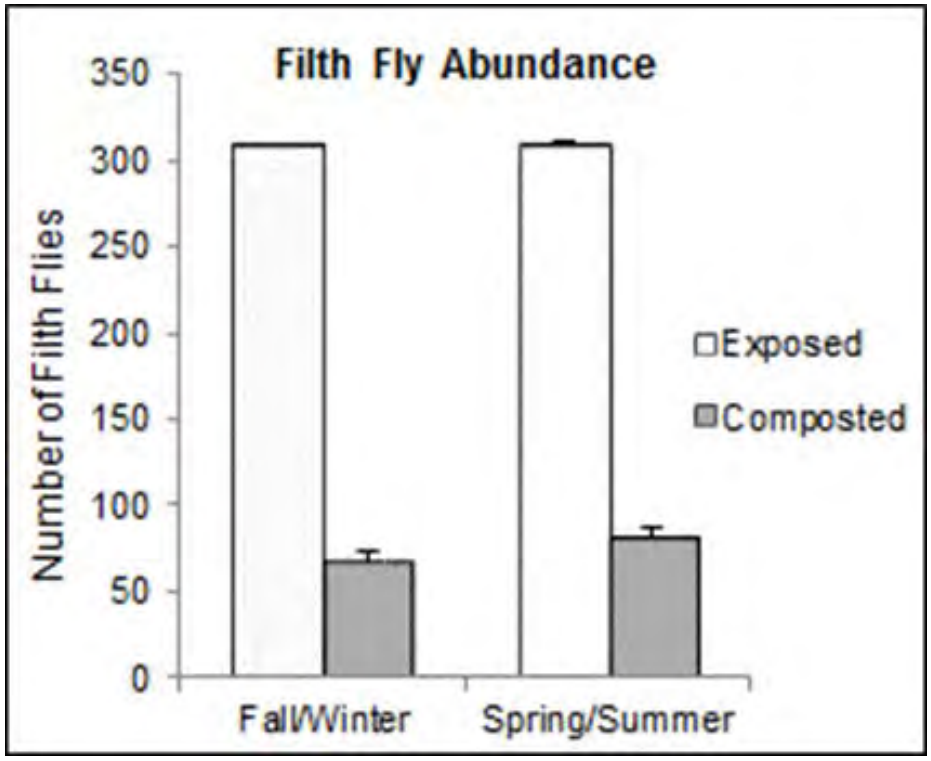

Figure 1 Total abundance of filth flies typically associated with carrion trapped near composted and non-composted beef cadavers throughout the entire period of decomposition (n=4). The number of filth flies at the exposed animal site was significantly higher than the composted site (p=0.0009).

There was a qualitative difference in the VOCs emitted from composted vs. exposed large rats. The only compound that was isolated from the composted rats was dimethyl disulfide, and it was not present in samples taken after day 6 (Table 1). The two compounds that were isolated from the exposed rats were dimethyl disulfide and dimethyl trisulfide, which were present on days 5, 6, 12, 41 and days 6, 12, 41, respectively (Table 1).

Table 1 Volatile organic compounds present in samples taken from exposed and composted rat cadavers.

	Exposed Animals						Composted Animals					
	Day 1	Day 5	Day 6	Day 12	Day 41	Day48	Day 1	Day 5	Day 6	Day 12	Day 41	Day48
	Fresh	*Active decay*			*Post decay*		*Fresh*	*Active decay*			*Post decay*	
Dimethyl sulfide	-	-	-	-	-	-	-	-	-	-	-	-
Dimethyl disulfide	-	*	*	*	*	-	-	*	*	-	-	-
Dimethyl trisulfide	-	-	*	*	*	-	-	-	-	-	-	-

The relationship between livestock production and filth flies is inevitable, and the considerable amount of waste, including animal mortalities, generated annually by these facilities can have a profound effect on filth fly populations. This study has shown that composting livestock cadavers greatly reduces the abundance of adult filth flies. While similar groups of flies commonly associated with carrion were recovered at both sites, the abundance was significantly reduced

at the composted carcass site. This study establishes that composting bovine mortalities greatly reduces filth fly abundance.

The composting process also decreases emission of VOCs produced by animal decomposition. Early in the active decay stages, unpleasant odor is mild, detectable only by persons in close proximity, and quickly diminishes. After observing very few flies in association with the composted carcasses, laboratory studies were initiated to examine fly olfactory cues emitted from composted carcasses using a smaller animal model. Specific VOCs emitted from animal cadaver compost have been studied for use as indicators of composting efficiency, and dimethyl disulfide and dimethyl trisulfide are also known to be olfactory cues for blows flies (Zito et al., 2014). Odors of decomposition that have been specifically tested using electroantennogram (EAG) techniques and elicited a strong response in blow flies include dimethyl disulfide, dimethyl trisulfide, and dimethyl tetrasulfide (LeBlanc 2008), and more recent work has included indole, isobutylamine and phenylacetic acid (Liu et al., 2016). Results from this study indicate that emission of select fly olfactory cues from a rat carcass is decreased when it is composted.

Conclusions

Regular and catastrophic animal losses create additional challenges for animal waste management. The methods of disposal that have historically been popular and environmentally sound, such as rendering and incineration, may not be cost efficient or as locally available, and alternative means such as composting are being explored. Composting is an economical, environmentally sustainable means of disposing of dead animal carcasses, and this study examined the associated insect activity as well as volatile organic compounds emitted from the compost pile. Composting livestock mortalities greatly reduces filth fly activity and inhibits propagation. Results from laboratory studies with volatile organic compounds released from exposed and composted animals indicate that the composting process may degrade or otherwise inhibit the release of important olfactory cues that typically attract flies to carrion. The breakdown or suppression of chemical cues may contribute to the overall decrease in filth fly activity associated with composted beef carcasses. Reduction in filth fly activity within the grounds of livestock facilities by composting mortality waste could also reduce the risk of flies contaminating the surrounding area by spreading pathogenic microorganisms acquired from the dead animals.

*All of this work should be cited from the following publication:

Dubie, T. R., J. L. Talley, J. B. Payne, A. W. Wayadande, J. Dillwith, C. Richards. 2017. Filth Fly Activity Associated with Composted and Non-composted Beef Cadavers and Laboratory Studies on Volatile Organic Compounds. Journal of Medical Entomology. 54: 1299–1304.

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Emergency Response: Composting in a Brucellosis Suis Outbreak

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June 2018 | Amarillo, Texas

EMERGENCY RESPONSE: COMPOSTING IN A BRUCELLOSIS SUIS OUTBREAK

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Written for presentation at the
6th International Symposium on Animal Mortality Management
Amarillo, Texas
Embassy Suites Hotel and Conference Center

Abstract. Emergency Response: Composting in a Brucellosis suis Outbreak

A Brucellosis suis outbreak was identified in early August 2016 on several small non-industrial farm in northeastern New York State. The disease was identified because a farmer became ill from the bacterium ***Brucellosis suis.*** The team of state veterinarians, an APHIS veterinarian and disposal people visited the largest affected farm to assess whether the pigs could be disposed of on the farm. There were a few places that could be used for composting however the farm was very wet and there were no staging areas to dispatch the pigs. The farms were also under a lot of stress with the outbreak and eventual loss of their pigs. In the end it was determined that the pigs should be hauled away from the farm, dispatched and composted.

Keywords. Brucellosis suis, disease outbreak, mortality composting, disposal.

In early August 2016, a Brucellosis suis outbreak was identified on several small non-industrial farms in northeastern New York State. The disease was identified because a farmer became ill from the bacterium **Brucellosis suis,** which was transmitted by the farms pigs.

Determining the area of the outbreak: The first batch of pigs were confirmed and disposed of at Cornell School of Veterinary Medicine in an alkaline digester. The NYS and APHIS vets assessed farms in the area to see how far the disease spread. As a number of farms surfaced with the disease the veterinarians checked to see if any of the pigs on the infected farms were recently sold or loaned to other farms. It was determined that a male intended for breeding had been sent to a farm in southern Maine. It had not been integrated into the herd so that pig was dispatched and composted in Maine.

A team of state veterinarians, an APHIS veterinarian and disposal people visited the largest affected farm to assess whether the pigs could be disposed of on the farm. At the beginning of the outbreak, pigs were being sent for rendering, however rendering company decided that they did not want to accept pigs that were associated with the outbreak and rejected the rest. There were a few locations that could be used for composting, however the farm was very wet and there was no staging area to dispatch the pigs. It was far to wet to consider burial. The farm was also under a lot of stress with the outbreak and eventual loss of their pigs, it would have been very traumatic to dispose of on farm. In the end it was determined that the pigs should be hauled away from the farm, dispatched and composted.

There was a farmer in the area that had experience composting livestock over the years, had equipment and was not raising pigs or other animals that could contract the disease. At this point it was late August, it was county fair time and it was hot. It was difficult to get haulers to transport animals because of the fair but transport trailers were hired after the fair activities and a team of vets from Massachusetts to Georgia were brought in to kill the animals and collect tissue samples, equipment and carbonaceous bulking material were secured. Eighty-five percent of the animals were dispatched by captive bolt, Piglets were chemically euthanized on the farms and transported to the compost site. Most animals were integrated into the windrow within minutes, piglets with in hours. Interesting note: There were few vets with experience using captive bolt and the vets that did have experience gained it from experience working in slaughterhouses. It was not common to find the captive bolt implement.

The site chosen on the farm was very protected by vegetation, .75 miles off a county road and away from the main farm. It was a location where dairy bedding was being stockpiled. Woodchips from a municipal landfill were trucked to the site. In this case, the chips were free but had to be conveyed about 100 miles to the site. Trucking was about $1000.00.

The processing of the animals occurred at two different times, August 17/18 and September 7. Containment was built and of the 270 pigs processed only one escaped for about 4 hectic minutes. A bed of woodchip 10 feet wide by 50 feet long and 20 inches deep was built on the soil pad. After each animal was dispatched it was conveyed to the pile with a leg chain attached to the bucket of a loader. Pigs generally have a higher percentage of body fat, so it is important to ensure that there is enough chunky,

absorbent carbon to allow air to circulate around the animals and absorb the excess fat.

Dead pigs were conveyed with a loader and placed in the second layer of the windrow.

Tissue samples were taken from animals to assess the extent of infection

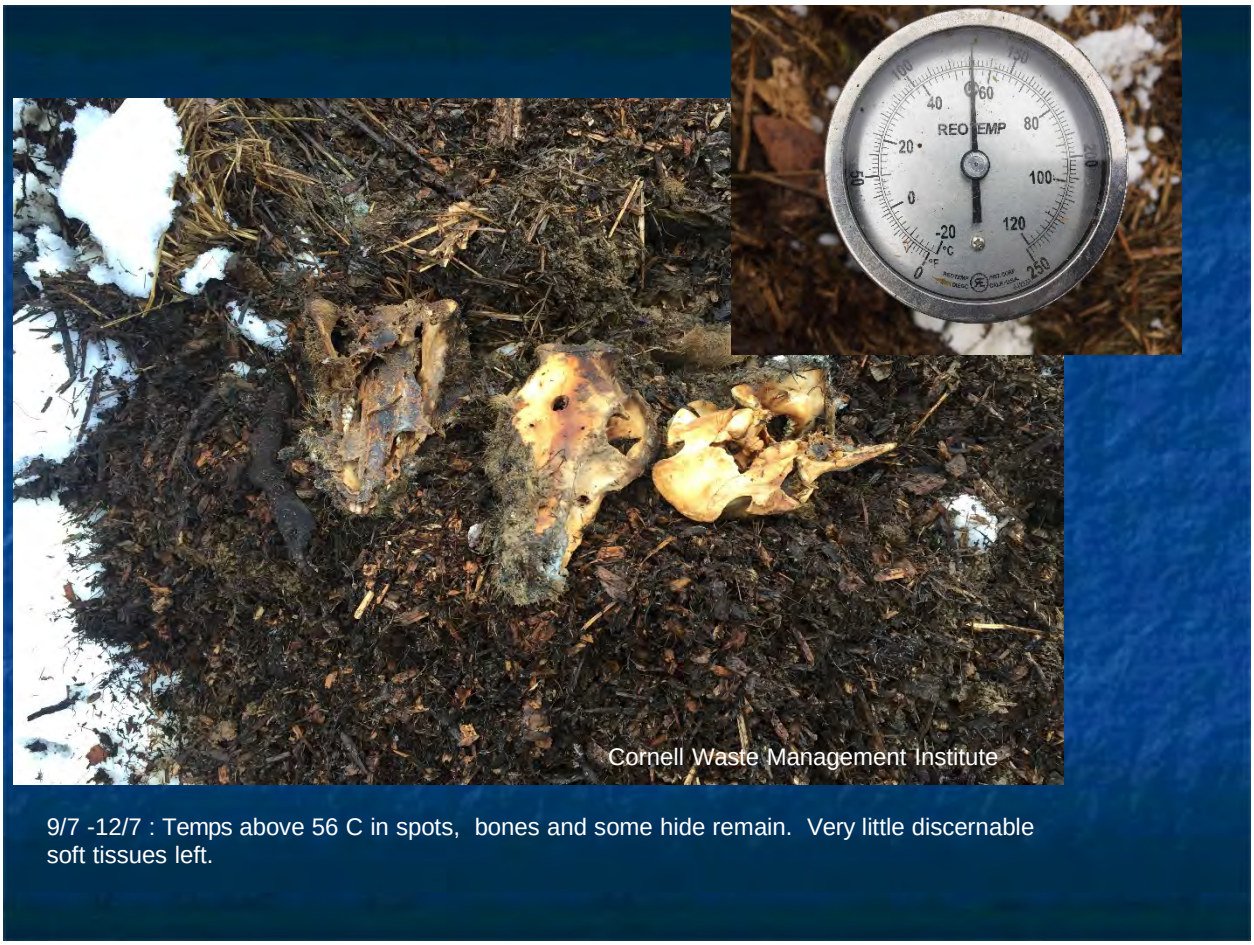
The animals were positioned in the piles, tissue samples were taken and a layer of carbon was placed over the dead stock. In the case of these pigs ranging from 2 to 1000 pounds, two layers of pigs could be stacked in the pile with carbon placed between the layers. If there were very large pigs on the bottom layer smaller pigs would be positioned on the next layer. The first layer had most of the large pigs because they are harder to

lift to a second layer. In the first build, there was a shortage of woodchips because of truck timing however, there was a large stockpile of dairy bedding mixed with manure. There was a good woodchip base where dead pigs were placed, and then woodchips were combined with bedding material for the second layer and overall cap. In the end, the two windrow dimensions were 13' wide x 6.5' tall x 65' long.

Temperatures were monitored to ensure that thermophilic temperatures above 133 degrees F were reached throughout the windrows.

Pigs													
Day/Date		1	2	3	4	5	6	8	10	12	14	9/28	10/ 12
Temp	18"	95	120	135	133	138	140	142	145	150	154	145	115
WR-1	36"	98	122	138	138	142	145	130	140	143	156	148	120
8/18-19													
WR-2	18"	97	125	133	135	139	140	152	152	152	150	150	148
9/7	36"	99	133	140	142	142	145	158	155	152	160	149	148

Ambient -95 F on 9/7 Opened pile on 12/7 ambient 30 F



Conclusions

The windrows were closed and the medium was left to finish the composting process, curing occurred throughout the winter. The compost process was successful in managing the Brucellous suis. Temperatures were monitored and recorded and indicated that temperatures needed to disinfect were met. After three months, soft tissue was gone; bone, teeth and some hide remained. Bones from young pigs disappeared while bones from mature pigs persisted. After the 3-month assessment, the windrows were left to finish the curing process. If the windrow is properly built with good natural air circulation, windrows can be, disinfected in as little as 7 days depending on the size of the animals and the targeted disease. There is still too much flesh in piles to really turn or move them. With livestock such as pig, it is not wise to move the windrow for about 3 months or when most of the flesh has been digested. It takes 9 months to complete the compost process in static windrows. Large bones can then be removed before land spreading.

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infected premises (IPs) and dangerous-contact premises (DCPs), or in a larger area if necessary, must be slaughtered immediately, whether they are obviously diseased or not (FAO, 2017). Owners should be asked to collect and confine their pigs the day before the slaughter team arrives. The animals should be slaughtered by methods that take account of animal welfare and the safety of operatives. The stamping-out approach require technology for animal carcass disposal as an integral component. According to FAO (2001), the carcasses of all pigs that die when there is an incursion of ASF should be disposed safely. This means disposal of the carcasses of animals that have been slaughtered or died naturally of the disease. It must be done in such a way that the carcasses no longer constitute a risk for further spread of the pathogen to other susceptible animals by direct or indirect means, for example by carrion eaters, scavengers or through contamination of food or water.

Fig 4: Decomposing pigs disposed in a bush

This is usually done by deep burial, depending on the nature of the terrain, level of water tables and availability of earth-moving equipment, or by burning, depending on availability of fuels and the danger of starting grass or bush fires (Fig 5).

Fig 5: Dead pigs set for disposal by burning in open air

If in situ disposal is not practical, it may be possible to transport carcasses in sealed vehicles to a disposal point. This should be done within the infected zone. It is not ideal, especially in countries such as in Nigeria where sealed vehicles for such purposes are not available and where vehicles in general are prone to breakdown due mainly to lack of maintenance culture and bad roads. If it must be done, provision should be made for an escort vehicle to disinfect any leakages and initiate salvage operations should the vehicle transporting the pigs develop technical problems or be held up. Whereas there are enabling statutory provisions that clearly stipulates the manner in which dead animal carcass shall be disposed in Nigeria, what is obtainable in practice is totally in contrast with the provisions of the statutes (Onyimonyi *et al*., 2013; Muhangi *et al.*, 2015; Jibril *et al*., 2016).Therefore, the country focuses much on prevention/control measures in the event of ASF outbreak.

Epidemiological Features influencing ASF Control/Eradication Strategies.

A number of epidemiological and other factors that favourably or unfavourably influence the strategies adopted and the ease of control/eradication of ASF in Nigeria have are as follows:

- Among factors that favourably impact on ASF control/eradication strategies include the fact that:
- It is an OIE listed viral disease
- High mortality and morbidity reaching 100 per cent
- It has no vaccine and no cure
- ASF is an emerging transboundary disease
- No domestic livestock species other than pigs is susceptible to ASF;
- Humans are not susceptible;
- *Ornithodoros* ticks that transmit ASF virus have not been described in Nigeria;
- ASF is a highly contagious and clinically apparent disease and disease recognition on the field should therefore be relatively easy.

  Those factors that are unfavourable to easy control/eradication include the facts that:
- The distribution of ASF in West Africa is not static and it is doubtful if some of the neighboring countries have adequate and effective early warning and early reaction capability to enable rapid detection and containment of the disease to the primary focus/i and eventual control or eradication. Therefore the threat of re-introduction of ASF into Nigeria from her neighbors remains high.
- In Nigeria, live pigs and pig meat are important means of spread of the disease. Scavenging and free-roaming pigs often seen feeding on village and abattoir/slaughter slab wastes and garbage play very significant role in spread of the disease among villages in Nigeria in the absence of the sylvatic cycle involving warthogs and *Ornithodorus moubata*.
- ASF virus is resistant to inactivation and may remain viable for long periods in fomites, infected pig tissues, meat and processed pig products;
- Many wild suid species and feral pigs are susceptible to ASF but may not develop overt disease;
- International, inter and intra state trade in live pigs and/or pig meat and products contribute to rapid dissemination of the virus in Nigeria;
- Although ASF is usually clinically apparent, it may be confused with other diseases by an inexperienced animal health personnel;
- Pigs that survive ASF infection may become carriers, although their role in transmitting the virus after about a month is uncertain; their tissues nevertheless remain infective for a period after active shedding has ceased;
- There is no vaccine available for ASF.

Strategies used for ASF Eradication in Nigeria

- In the absence of vaccines, the only available option for ASF eradication is stamping out by slaughter and disposal of all infected and potentially infected pigs. This is a proven method that has succeeded in eradicating ASF and other serious transboundary diseases.
- The main elements of a stamping-out policy for ASF are:
- Zoning of the country into infected zones, surveillance zones and free zones;
- Quarantine procedures to contain the disease, including pig-movement controls and prohibitions of the sale of potentially infected pig products;
- Enhanced epidemiological surveillance for ASF;
- Immediate slaughter of infected and potentially infected in-contact pigs, with prompt and fair compensation to owners;
- Safe burial or burning of carcasses and other infected materials;
- Cleansing and disinfection of infected premises;
- Keeping infected premises/villages without pigs for a safe period.

- Introduction of sentinel pigs for a period of sixty days before restocking.

Conclusion

This report examined methods of carcass disposal in Nigeria during major epizootics such as African Swine fever. Although there is no current reported case(s) of ASF in Nigeria as at the time of this report, our report show that in previous cases of ASF epizootics, the major methods of disposing carcass of dead pigs is by burying, burning or incineration. Other methods like composting, alkaline hydrolysis, licensed commercial landfill, biosphere process, etc are still under development.

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An Evaluation of the Efficacy of Composting as a Management Tool to Reduce the Viability of Newcastle Disease Virus

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June 2018 | Amarillo, Texas

An Evaluation of the Efficacy of Composting as a Management Tool to Reduce the Viability of Newcastle Disease Virus (*Rubulavirus*) in Egg Waste and Animal Bedding

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Written for presentation at the
6th International Symposium on Animal Mortality Management
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Abstract.

Between March 11, 2016 and June 1, 2016, a series of compost trials were conducted using wood shavings, poultry bedding and egg waste containing viable strains of the Newcastle Diseases Virus (NDV) to determine if aerobic windrow composting could be used as a management tool to deactivate NDV in egg waste. Prior to initiation of composting activities, samples of the egg waste were taken to validate the presence of live NDV strains. The study design consisted building two 15-cubic-yard volume piles that were constructed on two separate days, Pile #1 on March 11, 2016 and Pile #2 on March 14, 2016. Piles were built in accordance with existing United States Department of Agriculture (USDA) guidelines for emergency poultry mortality management, "HPAI Outbreak 2014-2015 Mortality Composting Protocol for Avian Influenza Inflected Flocks" (Miller et. al, 2015). Both piles were built in a similar fashion using the following methodology: first, a 12-foot wide by 12-foot long by 12-inch thick base of wood shavings was laid out, followed by the addition of 6 cubic yards of an equal mixture of egg waste and animal bedding.

Finally, a 12-inch thick cap of wood shavings was added on top of the mixture to provide insulation and odor control. Following initial construction, each pile was monitored daily for temperature, leachate activity, and odors for a 14-day period (Phase I). Daily temperatures were recorded at four fixed locations (North, South, East and West) at both 18-inch and 36-inch depths. At the end of Phase I, both piles were broken down and composite samples were taken for analysis to detect whether NDV was present. None was detected. Piles were then re-formed and water was added to achieve optimal moisture since the piles had become very dry over the course of Phase I. Piles were then monitored for an additional 14-day composting period (Phase II). On April 20, 2016, following the completion of Phase II, both piles were broken down and sampled once again for viable NDV, with no viable strains detected. At this point, the piles had completed the requirements of the USDA protocols and it was decided to combine them into a single pile. Water was liberally added as pile contents had, once again, dried significantly during Phase II. From this point, temperatures were monitored daily until June 1, 2016; leaving the pile undisturbed to finish curing. Based on the above, we believe that composting, when applied appropriately, may be used as an effective tool to manage waste materials containing active Newcastle Disease Virus strains.

Keywords.

Compost, feedstocks, wood shavings, animal bedding, egg waste, Newcastle Disease Virus, NDV, and, USDA HPAI Composting Protocols.

Background

Historically, facilities working with live animal disease strains have had to dispose of their tainted waste products by either landfilling, chemical inactivation, autoclaving or incineration (Guzmán and Feuerstein, 2010). However, threats to groundwater from burial practices, especially in areas with shallow water tables, along with air quality concerns and public health risks from incinerator emissions, have forced industry experts to seek suitable alternatives (Langston et al., 1997). The ultimate goal of any disposal scenario must include a plan that is cost effective, environmentally sound, and ultimately, protective of public health. Additionally, as more emphasis is placed on developing sustainable practices, it has become popular to direct reuse of discarded organic materials to agricultural settings where valuable crop nutrients may be reintroduced. In order to achieve this goal, it is important to ensure that animal diseases are fully deactivated prior to the agronomic utilization of the material.

Many pathogenic organisms that may persist in diseased carcasses and other waste-derived materials have been found to be inactivated by sustained periods of high temperatures in excess of 55 ^{o}C (Dougherty, 1999). In some cases, pathogens may be inactivated at even lower temperature ranges, such as Highly Pathogenic Avian Influenza (HPAI) Virus, which can be inactivated at sustained temperatures of 99 ^{o}F for 24-36 hours (Lu et al., 2003). In all cases, systems providing high heat for sustained periods have been sought out by disease outbreak responders. Composting offers such a solution by providing a high heat environment, through the aerobic, microbiological decomposition of organic ingredients and soluble nutrients, into complex organic compounds that are resistant to breakdown and subsequent leaching (Rynk, 1992). During the 2014-2015 outbreaks of HPAI in the mid-western United States, composting was one of the primary methodologies chosen to help manage the

millions of carcasses generated. Composting worked so well, that following the outbreak, a set of protocols entitled, "HPAI Outbreak 2014-2015 Mortality Composting Protocol for Avian Influenza Inflected Flocks" (USDA HPAI Protocols) were developed by the nation's leading compost professionals to help prepare for future events (Miller et al., 2015).

During the Spring of 2016, a Maine-based facility which currently conducts production activities that result in the development of vaccines for a host of various animal disease strains, approached the Maine Department of Environmental Protection (MEDEP) to have the Department help them develop a program to manage a series of byproducts generated during vaccination production, and which are currently being landfilled. These byproducts include: egg waste (shell, albumin, yolk, and non-viable chick embryos) and animal bedding from poultry cages (wood shavings, feces). In an effort to facilitate the exploration of sustainable alternatives to landfilling, facility staff partnered with the MEDEP to conduct composting trials. The trials were developed to address two specific questions: first, to determine if composting could be used to deactivate NDV strains present in the egg waste; and, second, to determine if the Facility could dispose of certain types of its organic wastes generated on-site in a sustainable way through composting. This paper focuses on results and observations noted during the course of the composting trials.

Materials and Methods

Study Site

Between March 11 and September 1, 2016, a series of composting trials were undertaken at the Elanco Animal Health Facility (Facility) located in Winslow, Maine. The site chosen for the composting trials is a small section of asphalt, measuring 40 feet wide by 150 feet long. The asphalt pad area was chosen as it provided a durable work surface in a remote location at the site. All surface water draining from the pad enters a grassy swale where potential leachate could be treated, if necessary, by filtering through moderately well-drained native soils (Figure 1).

Figure 1. Google Earth photo depicting study area. Composting site is located in lower center of image; note compost pile flanked on either side by tarped piles.

Pre-Composting

Prior to initiating composting activities, an inventory of the materials to be composted (feedstocks) was conducted to determine volumes of each material that would need to be composted. Specific materials included in our study were: wood shavings (soft wood), poultry bedding, and egg waste. Wood shavings were purchased locally from a soft wood supplier (pine/spruce). The poultry bedding consisted of wood shavings and poultry droppings. The egg waste consisted of whole eggs (shell, yolk, albumin) and non-viable poultry embryos from fertilized eggs. Both the egg waste and animal bedding were contained in compostable bags (EcoSafe 6400®). All of the waste materials were double-bagged for biosecurity purposes.

Samples of each of the raw feedstocks to be composted were collected for testing in order to ensure that proper nutrient and moisture values were accounted for in order to develop an optimal compost recipe. Each feedstock was analyzed for: Total Solids (%), Total Carbon (%), Total Nitrogen (%), Total Volatile Solids (%), Bulk Density (lbs./cu. yd.) and C:N Ratio. The results of this initial sampling appear in Table 1.

Table 1. Assessment of Raw Materials Used in the Elanco Composting Trials. All values reported on "As is Basis."

Parameter	Egg Waste	Animal Bedding	Soft Wood Shavings
Total Solids (%)	24.4	85.9	62.00
Total Carbon (%)	14.4	38.0	38.0
Total Nitrogen (%)	2.09	2.10	0.08
Total Volatile Solids (%)	23.4	79.4	44.5
Bulk Density (lbs./cu. Yd.)	1970	240	500
C:N Ratio	6.9	18.2*	452.3

*The animal bedding contained urine, feces and shavings.

Based on the combined analysis results, a recipe was developed that focused on maintaining an appropriate balance of carbon, nitrogen, water and unrestricted airflow to help initiate and sustain the composting process. Initially, it was decided to mix the egg waste and animal bedding in a 50:50 blend, in hopes of creating one homogenous feedstock. We soon found, however, that the blending was going to be complicated due to a facility requirement that both egg waste and animal bedding be packaged in compostable bags for biosecurity reasons. This required trial personnel to physically open most of the animal bedding bags to facilitate mixing. The Facility's front-end loader also assisted with puncturing the bags containing egg waste (Figure 2).

Figure 2. Initial mixing of egg waste and bedding material. The presence of compostable bags made homogenous mixing more difficult—Photo taken by Mark King.

Compost Pile Construction

Two trial piles were constructed on separate days (one on March 11, 2016 and a second on March 14, 2016) following the USDA guidelines for emergency poultry mortality management. Each pile was built in a similar manner: first, a 12-foot wide by 12-foot long by 12-inch thick base of wood shavings was laid out, followed by the addition of 6 cubic yards of an equal mixture of egg waste and animal bedding. Each pile was then covered with a blanket of 6 to 12 inches of clean wood shavings to retain heat, minimize odors, and serve as a barrier to vectors. During the trials, the piles were allowed to compost undisturbed for 14 days (Phase I). During that time period, average pile temperatures were monitored and required to meet 3 consecutive days at 131°F or 10 consecutive days at 110°F standard adopted for HPAI, to achieve desired pathogen reduction standards (Miller et al., 2015). Once Phase I temperatures had been met, the piles were turned and re-formed to compost for an additional 14-day period (Phase II), where the pathogen reduction standards were required to be met a second time. The piles were also covered with plastic “snow” fence to minimize wind erosion and provide an additional barrier to vectors. Figure 3 depicts the entire pile-building process.

Figure 3. Photos depicting the complete pile construction process: a) compost bed formation, b) addition of egg waste and bedding mix, c) addition of carbonaceous cap and d) application of snow fencing for vector control—Photos taken by Mark King.

Pile Monitoring and Compost Activity

Pile temperatures were monitored daily (at both 18 and 36-inch depths) using a pair of Reotemp® analog thermometer probes (300 Series) that were placed at each of four locations within each pile: north, south, east and west sides (Figure 4). In addition to temperature, observations were also recorded for odors, leachate and vector activity.

Figure 4. Final pile set-up, showing temperature monitoring points-note two separate thermometers located at each monitoring point (one at 18 inches in depth and one at 36 inches in depth)—Photo taken by Mark King.

Results and Discussion

Phase I vs. Phase II - Temperature Performance

Figure 5 shows the temperature performance of both piles during Phase I and Phase II of the composting trials. On Day #0, Pile 1 had a core temperature of 42ºF and Pile 2 had a core temperature of 47ºF. By Day #11, both piles had exceed 110ºF at both the 18- and 36-inch depths, but were unable to sustain those temperatures for 10 consecutive days nor reach 131 ºF for three consecutive days. Since neither pile was able to meet the 131ºF or 110ºF pathogen destruction standards, it was decided to hold off on turning each pile in favor of extending the Phase I in hopes of meeting the temperature standards. The lack of temperature response was most likely due to the continued problems with excessive pile dryness combined with the compostable bag remnants inhibiting microbial propagation and overall activity. Finally,

on Day 20, Pile 1 met the 110ºF for 10 consecutive days and it was turned, whereas Pile #2 met its 110ºF for 10 consecutive days standard on Day 21 and it was also subsequently turned.

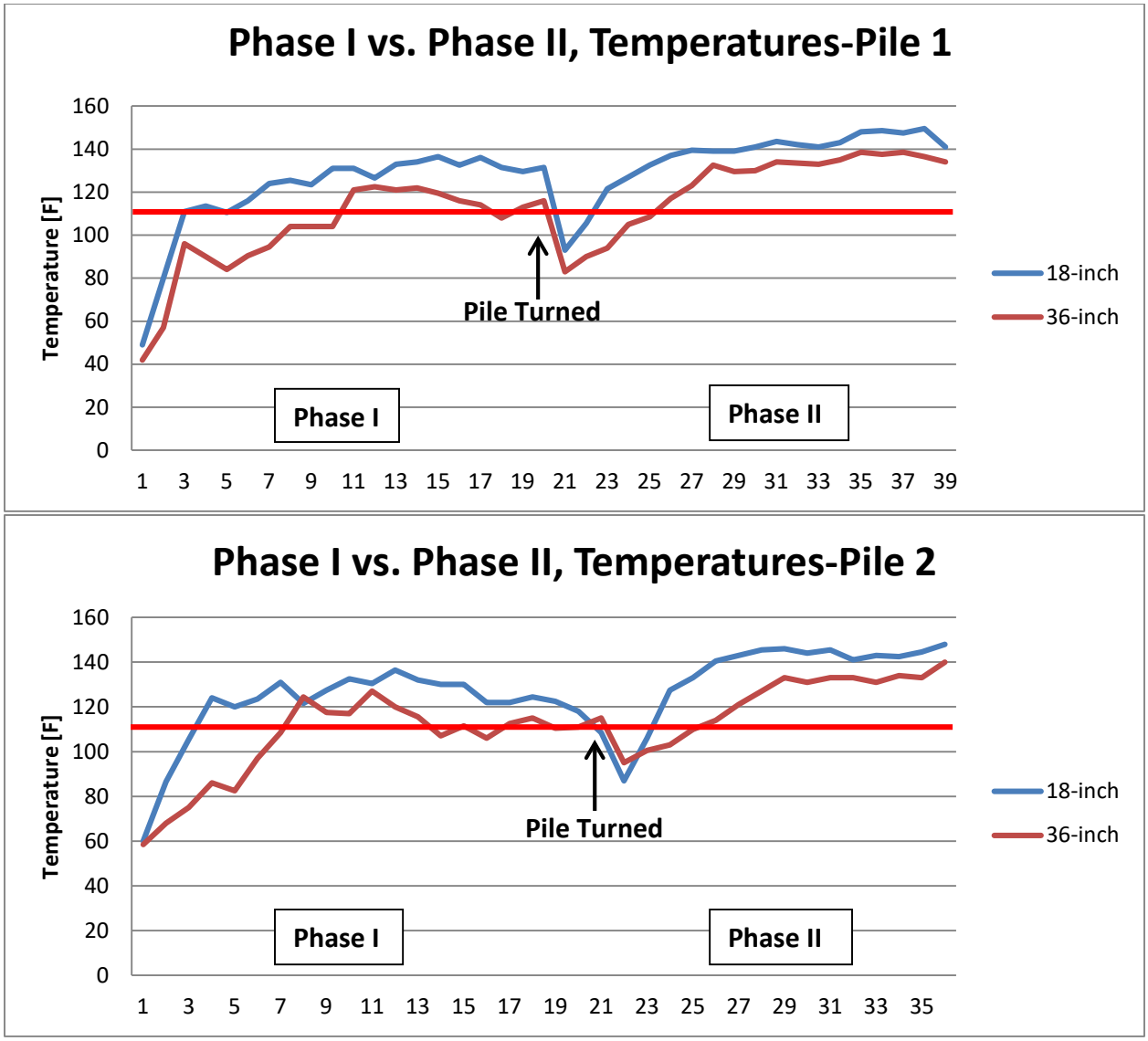

Figure 5. Comparison of temperature results for Pile 1 vs. Pile 2 during Phase I and Phase II. Note: Red horizontal line indicates 110ºF level.

Observations of the inner pile contents revealed that each core was very dry and moisture was liberally added to correct the deficiency. Prior to the water addition, pile contents were sampled for presence of viable Newcastle Disease Virus (Figure 6).

Figure 6. Sampling of compost after completion of Phase I for live New Castle Disease Virus—Photos taken by Mark King.

Piles were then mixed and re-formed (Figure 7). During the mixing process, water was liberally added to approximately 50-55% moisture (as measured by the “Squeeze Test”) due to the dry texture of the inner pile contents. Piles were then monitored for an additional 14-day composting period (Phase II).

Figure 7. Remixing of Pile 1 following completion of Phase I trials—Photo by Mark King.

Following remixing and the addition of moisture, temperatures appeared to rebound quickly in both piles during Phase II. By Day #12, Pile 1 had met the 131°F for three consecutive days pathogen reduction standard at both the 18- and 36-inch depths; whereas Pile 2 had achieved the standard two days sooner on Day #10. By April 19, 2016, both piles had successfully completed Phase II and were broken down and sampled for viable NDV. Piles were then remixed (Figure 8) and water was liberally added once again to regain 50-55% moisture due to excessive dryness of the mix. At this point, both of the piles had completed the requirements of the USDA protocols and it was decided to combine them into a single windrow (Figure 9).

Figure 8. Remixing of piles following completion of Phase II. Note the darker color in the pile material indicating advanced microbial decomposition—Photo taken by Mark King.

Figure 9. Photo depicting the "new" mega pile resulting from the merging of Piles 1 and 2—Photo taken by Mark King.

From this point on, temperatures in the newly formed pile were monitored daily until May 16, 2016, when it was opened and sampled for the presence of NDV. The windrow was then re-formed and allowed to continue curing on its own without further monitoring until September 1, 2016 when it was reopened for a final NDV sampling.

Newcastle Disease Virus Sampling

The egg waste used for the study was sampled and analyzed on March 11, 2016 prior to construction of the first compost pile to verify the presence of Newcastle Disease Virus in the material that would be tested in the compost trials. Additionally, composite compost samples were collected at the end of Phase I, the end of Phase II, and at the completion of the study to check for viable remnants of the NDV. A total of five samples were collected on the following dates: Pile #1 was sampled on April 1 and April 14, 2016; Pile #2 was sampled on April 2 and

April 19, 2016; and the combined pile was sampled on May 16, 2016. All samples were obtained by Carla Hopkins (MEDEP) using the following procedure:

- The top of the piles was "pulled back" using a front-end loader to expose the core of the pile.
- A sharpshooter shovel was used to bore into the pile at varying depths.
- Using a large stainless steel spoon, eight (8) discreet grab samples were obtained from the pile at varying depths and placed in a stainless steel mixing bowl. Note: care was taken to ensure that visible egg waste material was present in the sample.
- The sample was mixed in the stainless steel bowl until homogeneous.
- An aliquot of the compost was then removed from the bowl and transferred to a 500ml pre-cleaned plastic container (furnished by Elanco) using a stainless steel spatula scoop.
- The container was then covered and double-wrapped in plastic bags for transportation to the on-site laboratory.

Collected samples were analyzed and assayed by the facility's Quality Control Lab, using a standard Newcastle Virus Isolation Protocol.:

All of the samples returned negative findings for NDV, and thus demonstrated that composting may serve as an effective tool to deactivate live strains of the Newcastle Disease Virus.

Finished Compost Assessment

The finished compost pile was sampled on September 1, 2016, nearly six months since original pile construction; results appear in Table 2 below.

Table 2
Assessment of the finished compost generated from the Elanco Composting Trials. All values reported on "As is Basis."

Parameter	Finished Compost
Total Solids (%)	42.8
Total Carbon (%)	18.2
Total Nitrogen (%)	0.44
Total Volatile Solids (%)	37.1
Bulk Density (lbs./cu. Yd.)	380
C:N Ratio	41.4
pH	7.4
Conductivity	3.8

With the exception of a relatively high remaining C:N ratio of 41.4:1, the organic matter available in the compost would prove beneficial when used on nutrient depleted soils, although additional

composting using a supplemental nitrogen source would undoubtedly yield a more valuable soil amendment. The important fact to note is that when creating recipes for virus/microbial destruction, emphasis is placed on destruction of the pathogenic organism, not necessarily on the quality of the end product.

Odors, Vectors, and Leachate

No significant occurrences of odors, vectors or leachate were observed during the study. There were minor issues with flies/maggots and slight odors noted during the first few days following the Phase I turn, but this was short-lived once additional cover was placed over the offending areas.

Conclusions

The results of this study support the efficacy of composting as an effective method for deactivation of NDV. From an operational perspective, it was noted that egg waste alone did not provide adequate moisture content to sustain optimal composting rates. In addition, the compostable bags used to deliver the animal bedding and egg waste appeared to hinder the composting process as they did not readily break/rupture during initial mixing with a front-end loader, and did not show significant levels of degradation prior to temperatures of approximately 140°F or greater. If repeated on a long-term basis, more emphasis would need to be placed on developing a compost recipe that favors optimal moisture, C:N and porosity. This would best be accomplished by running multiple trials until a favorable response is achieved and replicated. Finally, odors, vectors and leachate were not significant issues as long as the management practices outlined in the USDA Mortality Protocol were followed.

Acknowledgements

The authors would like to thank their respective agencies: Maine Department of Environmental Protection and the Elanco Winslow facility, for their support and willingness to provide staff time to undertake this valuable effort. Additionally, the staff at the Elanco-Winslow facility provided excellent support, including equipment, manpower, and analytical services.

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Recent Demonstration Projects and the Field Application of Aboveground Burial for Carcass Disposal

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June 2018 | Amarillo, Texas

Recent Demonstration Projects on Above Ground Burial

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Abstract. *Carcass disposal continues to play a critical role in the effective management of animal disease outbreaks and natural disasters impacting agriculture. To explore the possible benefits of aboveground burial over traditional burial methods, our team conducted a demonstration project between 2015 and 2016 to optimize, evaluate, and operationalize aboveground burial as an alternative to existing large animal carcass disposal methods. The system design included a shallow trench excavated into native soil, a bed of carbon material and a layer of animal carcasses. Excavated soils are subsequently placed back in the trench forming a mound on which a vegetative cap is established. Finally, the perimeter of the mound was trenched to prevent the intrusion of surface water into the system. Based from the results from this project, aboveground burial was implemented to dispose of sheep infected with Foot & Mouth Disease in Tunisia in 2017. The presentation will discuss lessons learned from the demonstration project and the actual application of this method in the field.*

In addition, the presentation will include information on three similar demonstration projects that will be conducted by the same research team. The first project will be conducted in Texas in conjunction with the 6th International Symposium on Animal Mortality Management. This project will directly compare carcass decomposition with the aboveground burial, traditional deep burial, aerated static pile composting, and conventional windrow composting.

The second project is funded by a grant from the Natural Resources and Conservation Service (NRCS). This project will compare nutrient migration and carcass decomposition between traditional burial and aboveground burial in both sandy soils and heavier clay soils.

The third project, funded by the United States Department of Agriculture, Animal & Plant Health Inspection Service, addresses the issue of pathogen viability in the AGB environment.

Keywords. Above ground burial, demonstration project, foreign animal disease, natural disasters, carcass management

Introduction

Environmental impacts from carcass disposal during catastrophic events are a significant concern globally. Despite a history of costly, ineffective, and environmental-damaging carcass disposal efforts, large animal carcass disposal methods have advanced little in the last decade. A catastrophic loss of livestock due to disease or natural disaster today will likely be managed with the same carcass disposal techniques used in previous decades and will likely result in the same economic, health, and environmental impacts.

Above Ground Burial Demonstration Projects

Technical approach, Virginia field test 2015

In April 2015, researchers in Virginia, United States, conducted a field test to assess the environmental impact and effectiveness of Above Ground Burial (AGB) in decomposing livestock carcasses. Figure-1 shows a basic diagram of the AGB technique. The four designs evaluated included shallow trenches excavated into native soil to a depth of between 18 and 28 inches. Eight inches of loose soil or carbonaceous material were placed on the bottom of the trench, followed by a single layer of animal carcasses as seen in Figure 2. Excavated soils were subsequently placed on top of the animals forming a mound on which the vegetative layer was established. The site was visited weekly for the 1st month following installation and then once per month thereafter for the next year. Visual observations and pictures were taken to document the results. 1 year after the ABG field test was initiated, investigators excavated to the bottom of each design to assess the extent of carcass degradation and to collect soil borings for subsequent analysis. The assessment was simply a visual observation of degradation. In addition, soil samples were collected using a soil auger at depths. Each sample consisted of a single core from each of the four treatments and two samples from adjacent areas where animals were not buried. Soil samples were analyzed for total N, ammonium N, nitrate N, mehlich P, and pH.

Figure 1. Above Ground Burial Cross Section

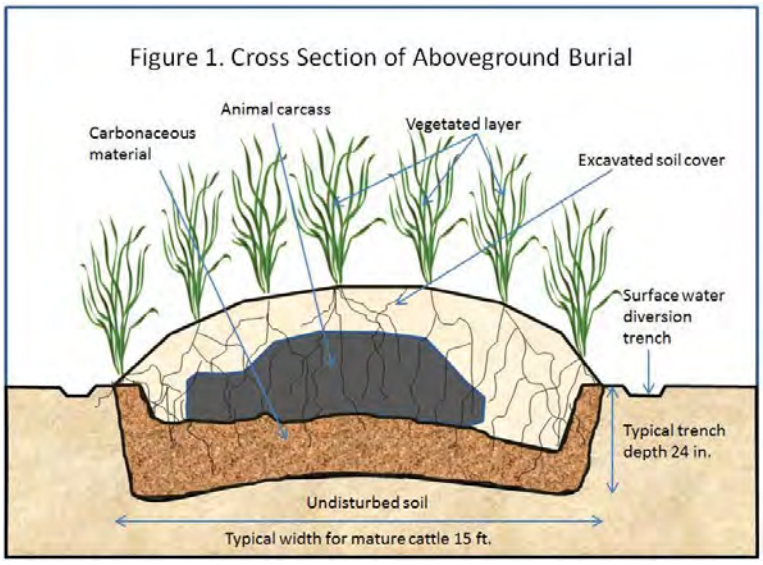

Figure 2. Cows placed within the trench

Figure 3. Vegetated Above Ground Burial system

Technical approach, Tunisia 2017

After the seizure of 151 sheep from the illegal trade by the Tunisian customs authorities, the animals were placed in a quarantine center. For the duration of a week, clinical signs were observed, and tissue and blood samples were taken to be analyzed for infectious diseases. The results of these tests showed that serological traces of the FMD virus using the 3 ABC test with Peste des Petits Ruminants Virus (PPRV) and Bluetongue Virus found. Based on these results, the decision was made to depopulate and dispose of the herd.

The team chose the AGB method for the disposal of 111 of the 151 animals because it appeared to be an easy method for implementation with a low cost of execution. 40 sheep died from PPRV and were managed through traditional burial. Initially, the animals were to be slaughtered and buried on the site of the quarantine center but were ultimately the disposal site was relocated a more remote location with clay soils.

The team excavated a trench 27 inches wide by 60 inches deep and 230 feet long and added about 12 inches layer of straw at the bottom of the trench. Next, they placed 111 sheep carcasses (total weight of approximately 9400 pounds) side-by-side in a longitudinal position and then covered them with 60 inches of excavated soil. Finally, they seeded alfalfa on the

mound. Based on the size of the trench and the number of animals, the AGB method resulted in a carcass density of 4.66 f2 per carcass.

Figure 4. Above Ground Burial Trench in Tunisia

Technical approach, Amarillo, Texas field test 2017

As part of the field demonstrations at the 6th International Symposium on Animal Mortality Management in Amarillo, Texas, an AGB treatment was installed in December 2017 and will be excavated and evaluated on during the Symposium. This demonstration is part of side-by-side demonstrations of above ground burial, traditional burial, static pile composting and aerated static pile composting.

Figure 5. Above Ground Burial Demonstration in Amarillo, Texas

Technical approach, Virginia field test 2018

Two additional demonstration projects funded by the Natural Resources Conservation Services (NRCS) are being conducted in Virginia in 2018. The first project was installed in January 2018 with cattle in clayey soils in the Shenandoah Valley of Virginia. The system at the second site was installed with swine in May 2018 in the sandy soils of the Tidewater region of Virginia. These projects will collect additional data to better understand the impact that this technique on water quality by collecting data on the movement of both nitrogen and phosphorus into the deeper soil profile. Additionally the project will compare the two above ground burial treatments with data collected from a traditional burial pit. The project will also compare nutrient migration between the coarse (sandy) and fine (clay or clay loam) soils.

Figure 6. Shenandoah County site

Figure 7. Tidewater, Virginia site

Figure 8. Tidewater, Virginia demonstration project with erosion control matting

Technical approach, Virginia field test 2018, Pathogen Inactivation

In coordination with the Tidewater, Virginia project and through a grant from the Animal, Plant Health Inspection Service (APHIS), the research team will evaluate the effectiveness of the AGB method for the inactivation of animal pathogens. Previous demonstrations have shown the method to be effective for carcass decomposition but to date, no data has been collected on its effectiveness in inactivating pathogens. This research will use 40 pound feeder pigs.

The fate of clostridium perfringes will be monitored. This system will be installed in June 2018.

Results

Virginia field test 2015

One year after installation, on June 9, 2016 investigators excavated to the bottom of each design to assess the extent of carcass degradation and to conduct borings for subsequent analysis. In designs 1 through 3, carcass degradation was approximately 95% with only the larger bones remaining. Carcass degradation in design 4 was only around 60% with some flesh, hide and fatty tissue remaining. The most significant difference between designs 1 through 3 and design 4 was the depth of the trench. The depth of designs 1 through 3 was 18 inches or shallower while design 4 was 28 inches deep. The deeper design contributed to an anaerobic environment in the trench which inhibited the biological activity found in the shallower designs. The anaerobic conditions of design 4 were more comparable to traditional burial methods and resulted in only partial decomposition of the carcasses.

Soils from various depths beneath each design and in two background locations were sampled and analyzed for nutrients to assess the vertical migration of nutrients into the soil profile. Phosphorus levels decreased with depth and were consistent with the background samples. These results were expected based on typical phosphorus movement through the soil profile. Like phosphorus, nitrogen concentrations decreased in the first 24-inches below the bottom of each trench. Nitrogen components, which are typically more mobile than phosphorus in soil, do not appear to have leached greater than 24 inches below the bottom of each trench. The elevated concentration of nitrogen was in the ammonium form and there did not appear to be any elevated concentration of nitrate nitrogen. We presume that we did not get nitrification/denitrification in the trenches. There are still many questions about the fate of nitrogen from these animal carcasses.

Tunisia 2017

The AGB mound was completed on May 05, 2017. The following observations were made 1 week later:

- Absence of liquid from the ditch
- Absence of odors
- Absence of cracks
- Absence of flies.

On June 2, 2017, 4 weeks after AGB pit completion, only a few cracks were observed. Due to a lack of precipitation, the alfalfa had not yet germinated. The overall cost of the Tunisia operation did not exceed $300 US.

In April 2018, the AGB system was excavated to evaluate carcass decomposition. Only wool and larger bones remained within the system.

Figure 8. Above Ground Burial System in Tunisia

Figure 9. Excavated remains in Tunisia at 1 year

Amarillo, Texas field test 2017

The system will be excavated and evaluated on June 6, 2018 during the 6th International Symposium on Animal Mortality Management.

Virginia field test 2018

The Shenandoah County site will be evaluated in November 2018 and the Tidewater site will be evaluated in April 2019. Soil sampling for nutrient migration will be conducted as part of the site evaluations.

Virginia field test 2018, Pathogen Inactivation

Samples will be collected and analyzed throughout the summer and fall of 2018.

Conclusion

Based on the analysis conducted to date, AGB appears to offer many benefits over traditional burial for catastrophic mortality management. Application of this method to manage sheep infected with FMD, bluetongue, and sheep plague in Tunisia demonstrated its practicality in that environment. Site design will be critical to the success of this option. Soil characteristics and depth to groundwater are the key parameters to consider to ensure minimal environmental impact. Pathogen inactivation in this environmental remains a key question that needs to be answered. Ongoing research will help answer this question and determine the limitations of the method and its application during large disease outbreaks or natural disasters is appropriate.

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Consulting and Operating Services for Organics Processing

The NaturSoil Company has been in business since 1975 providing management and consulting services for beneficial use of organic residuals in the wastewater, solid waste and livestock industries. Clients include governments, APHIS, slaughterhouses, wastewater treatment facilities, livestock operations, solid waste processing companies, military installations and horticultural enterprises.

NaturTech Containerized Composting Systems for Organics Processing

Renewable Carbon Management LLC designs and builds containerized composting and biofertilizer manufacturing systems using various patents owned by its founder, Jim McNelly and associated proprietary technologies.

Technical Breakthrough

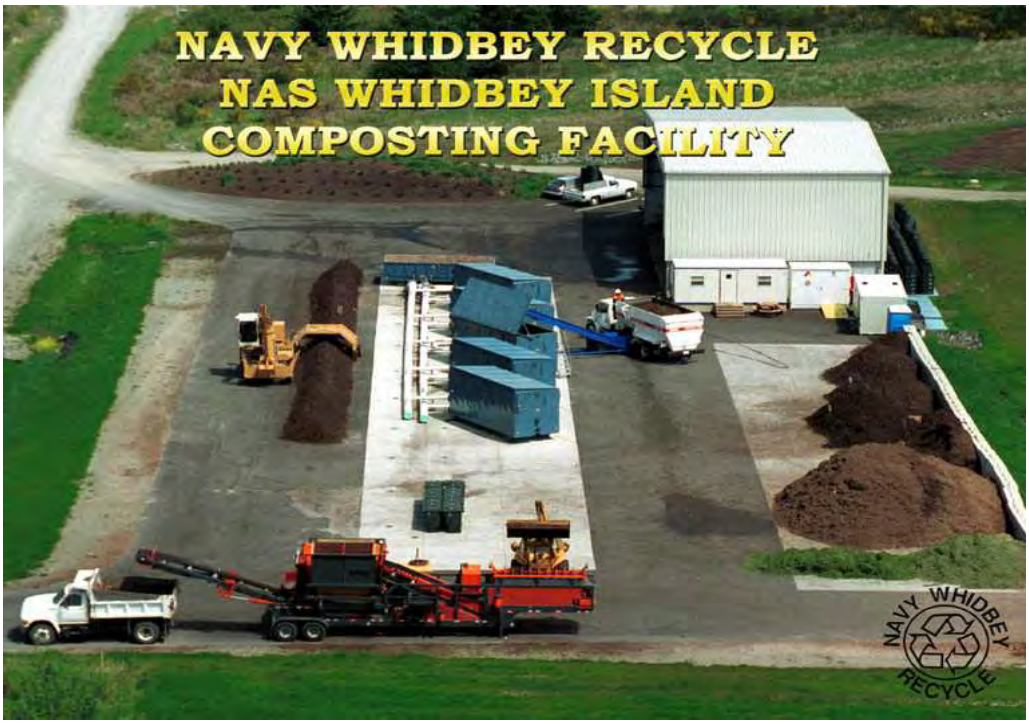
RCM has invented and patented a containerized method of processing virtually any organic material at a 10-1 C/N ratio and manufacture a 4% N stabilized fertilizer, and do this in a way that eliminates loss of N to ammonia or nitrates during stabilization. A 4% N stabilized fertilizer is a major breakthrough in nutrient delivery to plants as well as a solution to NOX air pollution caused by ammonia release and nitrates in ground and surface water. Biofertilizer manufacturing, however, changes the industry since the value of the biofertilizer is such that waste disposal fees are no longer essential for a processing facility to be profitable. A 4% N stabilized fertilizer has never previously existed on a commercial scale.

NaturTech Composting System in Rio Rancho, New Mexico


NaturTech 40’ bio-container for capturing ammonia

Type II Containers

Replaceable Wear Liner


NAVY WHIDBEY RECYCLE
NAS WHIDBEY ISLAND
COMPOSTING FACILITY
NAVY WHIDBEY
RECYCLE


Underside of Water-tight RCM Bio-container



Efficacy and Efficiency of Poultry Carcass Composting Using Different Mechanical Mixing Equipment for AI Outbreaks

Jennifer Keaten, University of Iowa

June 2018 | Amarillo, Texas

Efficacy and Efficiency of Mechanical Mixing Equipment for Poultry Carcass Composting for Avian Influenza Outbreaks

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Written for presentation at the
6th International Symposium on Animal Mortality Management
Amarillo, Texas
Embassy Suites Hotel and Conference Center

Abstract.

There are several disposal methods of AI infected poultry carcasses available in the U.S., which include on-site burial, landfill, incineration, rendering, and composting. Of these methods, composting is the most environmentally friendly and poses low risk for biosecurity. The United States Department of Agriculture (USDA) has developed a comprehensive plan for composting AI infected carcasses. The current protocols have the potential for areas of anaerobic pockets within the windrow due to inadequate mixing and the large carcass size of whole birds. This could lead to ineffective virus neutralization or prolonged composting times and higher resource costs. The purpose of this project was to determine if using a horizontal mixer wagon to mix composting ingredients or a vertical mixer wagon to mix and cut up the compositing ingredients is an economical

and timely means to accelerate the tissue break-down and obtain optimal temperatures for poultry carcass composting during an AI outbreak. A replicated trial with 3 treatments, Horizontal Mixer (HM), Conventional Layering (CL) and Vertical Mixer (VM), and three replications was initiated at the Compost Research and Education Center part of the University of Maine Forest and Agricultural Experimental Station called Highmoor Farm. Daily temperatures and screened core sample weights (screen weights) on day 0, 16, and 30 were recorded for each of the compost piles. The time to build each replication was recorded and used to help calculate the cost of each method. Data on equipment, carbon material and labor costs was collected from private contractors from the 2014-2016 HPAI outbreak and used to compare costs between methods. All treatment methods reached USDA protocol temperatures to neutralize the HPAI virus. Screen weights for both the VM and HM treatments were lower than the CL treatment. Screen weights decreased significantly from day 0 to day 16 for the VM and HM treatments with no significant change from day 16 to day 30. When comparing costs, the mixer wagon methods were more cost effective than the CL method when using high volume equipment. The data from this study supports the use of a mixer wagon to reduce particle size and mix ingredients for more timely and effective composting of poultry carcasses.

Keywords. carcass management, compost, high pathogenic avian influenza, poultry

Introduction

There are several disposal methods of poultry carcasses available in the U.S. It is important that the disposal of choice be timely, cost-effective, biosecure, environmentally friendly and have a positive public perception. Disposal options include on-site burial, landfill, incineration, rendering, and composting. In the past, on-site burial was the most common method of disposal, but concerns with ground water contamination and public perception caused it to fall out of favor (Swayne and Akey; Blake and Donald). It also raises concerns about prolonged survival of the virus and slow decomposition of the carcasses (Graiver et al.). Landfill burial was the primary method used for the 2002 LPAI outbreak in Shenandoah Valley, Virginia but was associated with high transportation fees as well as biosecurity risks with removal of the carcasses from the site as well as high tipping fees ranging from $45 to $140 per ton (Swayne and Akey). Groundwater contamination is also a concern with landfill disposal (Swayne and Akey). Incineration comes with high fuel costs, concerns for air pollution and smoke complaints, high transportation costs and biosecurity concerns with moving the infected carcasses off-site to the incinerator (Swayne and Akey). Rendering is another option, but due to biosecurity concerns, few rendering facilities will take infected carcasses (Swayne and Akey). Finally, composting is a means of virus neutralization and was used during the 2002 Shenandoah Valley LPAI outbreak when concerns about ground contamination, pollution and biosecurity was rising with other methods (Swayne and Akey). Of all these methods, composting is the most environmentally friendly of the options and poses the lowest risk for biosecurity.

During the 2002 LPAI outbreak in Shenandoah Valley, Virginia, improper construction of windrows raised concern that larger carcasses, such as market weight turkeys, could not be effectively composted. However, in 2004, during an outbreak on the Delmarva Peninsula in Pennsylvania, composting was successfully used with 5 pound broilers to control the spread of

the virus (Tablante and Malone). This led to research in 2004 in Virginia with 40 pound market weight turkeys that confirmed composting is successful if done properly (Bendfeldt, Peer and Flory; Flory and Peer). The Virginia research also showed that crushing, shredding, or tilling of the carcasses can speed the degradation and optimal temperatures by opening the carcasses and releasing and distributing moisture, increasing surface area to volume ratio, and exposing the bones to decomposition. Temperatures reached 140° F within 5 days for crushed carcasses and 16 days for whole carcasses. Furthermore, whole birds tended to roll off the piles more, necessitating more labor to replace them in the pile and more carbon material to cover them (Bendfeldt, Peer and Flory).

At an Iowa layer operation infected with HPAI in 2015, particle reduction size and mixing of carcasses and carbon material was successfully utilized to compost more than 4 million birds. Initially, a horizontal tub grinder was used to grind up carcasses and carbon material. Then, a Tebbe manure spreader, with the horizontal spinners off and at a very low discharge speed, created the compost windrows (Elbert). The tub grinder was used inside of a manure shed and loaded with birds, corn stover and wood chips and the mixture was loaded into the manure spreaders and taken to the outdoor composting site. During the height of the operation, when the crew was running most efficiently, a crew of 3-4 five-cubic yard loaders, 1 tub grinder, 1 tractor with a 42-cubic yard Tebbe manure spreader and 1 tractor with a 32-cubic yard Tebbe manure spreader could process approximately 350,000 birds in 12-13 hours (Elbert).

The USDA has developed a comprehensive plan for composting AI infected carcasses titled “Mortality Composting Protocol for Avian Influenza Infected Flocks” (Miller et al.). This plan requires that all carcasses, feed and litter be composted in windrows for 28 days prior to release of the material from the site. The windrows must reach an average of 131° F for 3 consecutive days during the first 2 weeks, at which point, the windrows are turned and then must reach 131° F for 3 consecutive days during a second 2-week period. Alternatively, if 131°

F is not reached, 110° F for 10 consecutive days during both 2-week periods is acceptable. The provided protocols do not currently support the use of mechanical equipment that aggressively mixes or grinds due to concern with virus aerosolization. The current protocols have the potential for areas of anaerobic pockets within the windrow due to inadequate mixing and large carcass size of whole birds. This could lead to ineffective virus neutralization or prolonged composting times and higher resource costs.

If an economical and safe means for carcass size reduction and mixing can be accomplished, then, in theory, the decomposition and pile temperatures will be more uniform and, therefore, virus inactivation and carcass degradation will occur faster. If it is established that carcass reduction and mixing is more effective at composting carcasses, then the question must also be addressed if there is an economical and time effective means to accomplish this in a large outbreak situation. The purpose of this project was to determine if using a horizontal mixer wagon to mix composting ingredients or a vertical mixer wagon to mix and cut up the compositing ingredients is an economical and timely means to improve and accelerate tissue break-down and obtain optimal temperatures for poultry carcass composting during an AI outbreak.

Materials and Methods

Layer Hens Carcass Composting Trials

On August 8th, 2016, a replicated trial with two treatments, horizontal mixer (HM) and conventional layering (CL), and three replications was initiated at the Compost Research and Education Center part of the University of Maine Forest and Agricultural Experimental Station called Highmoor Farm. The six piles were oriented in a south to north direction on a paved surface. All feedstocks were handled by a tractor loader with an approximately ¾ cubic yard bucket. An 18-inch base layer of used horse bedding, moistened slightly with water,

approximately 6 feet wide and 30 feet long was formed for each treatment. Feedstocks for each replication were; used horse bedding, wood chips, poultry manure and chicken carcasses. The average number of birds per bucket load was 254 birds, which was determined by counting 12 bucket loads of birds on 3 different occasions.

Pile formation was different for each treatment. For the HM treatment one bucket of used horse bedding, one bucket of wood chips, one bucket of poultry manure, one bucket of chicken carcasses and 100 gallons of water were loaded in the HM (Kuhn Knight Model 3042) and allowed to mix. Mixing occurred continuously as feedstocks were added. This mixture was discharged to the top of the 18-inch base layer of used horse bedding. For the CL treatment feedstocks were layered directly on to the base layer in the following order; a ½ bucket of chicken carcasses, 1 bucket of used horse bedding, 1 bucket of poultry manure, 1 bucket of wood chips, another ½ bucket of chicken carcasses, and another bucket of used horse bedding. Each layer was moistened with water as needed. Finally, both treatments were covered with an approximately 10 to 12-inch layer of dry wood shavings for vector control. All six piles were approximately 5 feet in height and 8-10 cubic yards including the cap and base material.

All piles were created with one person operating both the tractor and HM and the time to create all piles, except pile 1, was timed for comparison. In accordance with USDA protocol, two back connect bimetal thermometers (Reotemp®) were placed 18 inches deep and 36 inches deep in each pile. (Miller et al.). The thermometers were placed on the east side of piles 1,3,4,6 and the west side of piles 2 and 5.

Temperatures were recorded manually once a day (Monday-Friday, excluding holidays) for a 30-day period. USDA protocol allows turning of HPAI piles after 14 days if temperature requirements are met, so on Day 16 all treatments were turned with the tractor loader by first rolling the pile over to the east and then rolling back to the west to their original location. A 2-gallon bucket sample of the core was taken from both the east and west side of the piles on day

0, after turning on day 16, and on day 30. All pavement was marked with orange spray paint for the start and stop of each pile and sample locations were 5 feet from the edge of the pile markers to avoid sample bias. Samples were screened through a ½ inch mesh screen. The remaining material that did not pass through the screen was weighed and recorded. These measurements were referred to as screen weights.

On August 31, 2016, a third treatment, vertical mixer (VM), was created including the same feedstock materials as in the HM and CL treatments. However, the VM treatment did not have water added to the mixer or the base layer when first created. These piles were created west of the HM treatment on the paved surface. A base layer was created in the same manner as the previous treatments. One bucket of chicken carcasses, 1 bucket of used horse bedding, 1 bucket of wood chips, and 1 bucket of poultry manure was placed inside a VM (Kuhn Knight Vertical Maxx VT144) and allowed to mix. The VM was used to apply the mix to the top of the horse bedding. Due to the low discharge door on the VM, the first load was applied to the entire length of the base layer rather than as an individual pile. The next 2 loads were mixed similarly and applied across the length of the base layer again. Finally, the piles were covered with a cap in the same manner as the previous treatments. The VM piles were not as tall as the HM and CL piles, but were wider, due to the low discharge door of the VM, and were approximately 3-4 feet tall and a total of 8-10 cubic yards including base and cap.

Thermometers were placed in the VM treatment and temperatures were recorded once daily for a 28-day period as in the HM and CL treatments. The piles were turned in the same manner on day 14 and day 28. On day 14, the VM replications were split open with the tractor loader and approximately 100 gallons of water was added to the center of the piles due to low moisture content. On day 0 and after turning on day 14 and 28 core compost samples were collected, screened, and weighed as described for the previous treatments.

Screen weight data for each treatment from day 0 and after turning (day 14 or 16 and day 28 or 30) were compared using independent t-tests with Microsoft Excel software. Variances for each data set were calculated and either an equal or unequal variance independent t-tests were used depending on the variance ratio between treatments.

Economic Calculations

For each of the treatment methods, the time to create the replications was recorded. The average number of birds per bucket was used to calculate the time it would take to process 200,000 birds with each method. Due to the small scale of our operation, calculations were then extrapolated for larger sized equipment that could handle more birds at the same time. It was assumed it would take the same amount of time to process the higher amount of birds with larger equipment, more equipment, and an operator for each piece of equipment.

Equipment and cost information was collected from the 2015 HPAI outbreak in Iowa. Information included hourly rates for equipment (including operator, equipment, and fuel), equipment type, number of each piece of equipment and average number of birds processed in a day (Elbert).

Cost calculations were made for composting of birds for layer barns and turkey barns based on estimated times for each treatment, equipment numbers and cost information from the Iowa layer farm outbreak (Elbert) and the Iowa State University 2016 Iowa Farm Custom Rate Survey (Plastina, Johanns and Erwin), carbon amounts from the treatments, and carbon amounts and costs from recent HPAI outbreak (Payne). Based on recommendations from a USDA agricultural economist, a low and high range is provided for changes in supply and demand depending on the availability of equipment, labor, and carbon material in different regions of the country and on the scale of the outbreak (Johnson). Since data was provided from actual HPAI outbreaks, the high range is 1.25 times the low range, rather than the suggested 1.5 times the normal cost.

Results

Temperatures

Both the HM and CL treatment temperatures (Figure 1 and 2, respectively) performed as expected for appropriately formed compost piles. The HM treatment temperatures reached above 131^{o} F for both the 18” and 36” depth by day 4, which was 1 day sooner than the CL treatment at day 5. The HM treatment 36” temperatures were approximately 10 ^{o} F warmer than the CL treatment 36” temperatures during the first 14-day cycle. The temperatures for the VM treatment (Figure 3) only reached 131^{o} F at both the 18” and 36” depth for one day after the piles were watered and turned. The VM treatment was significantly dry compared to the other treatments. Additionally, the VM piles did not have a sufficient parabolic shape, as is ideal for composting, due to the low discharge door on the wagon used in our trial. The temperatures for the VM treatment did stay above 110^{o} F for most of the treatment trial. The thermometers for the VM treatment were reset on day 12 of the first cycle due to the thermometers sinking too low into the pile and reading close to ground level at 36” level. The reset resulted in a 10^{o} F increase in temperature for the 36” reading and a 3 degree increase at the 18” reading (Figure 3).

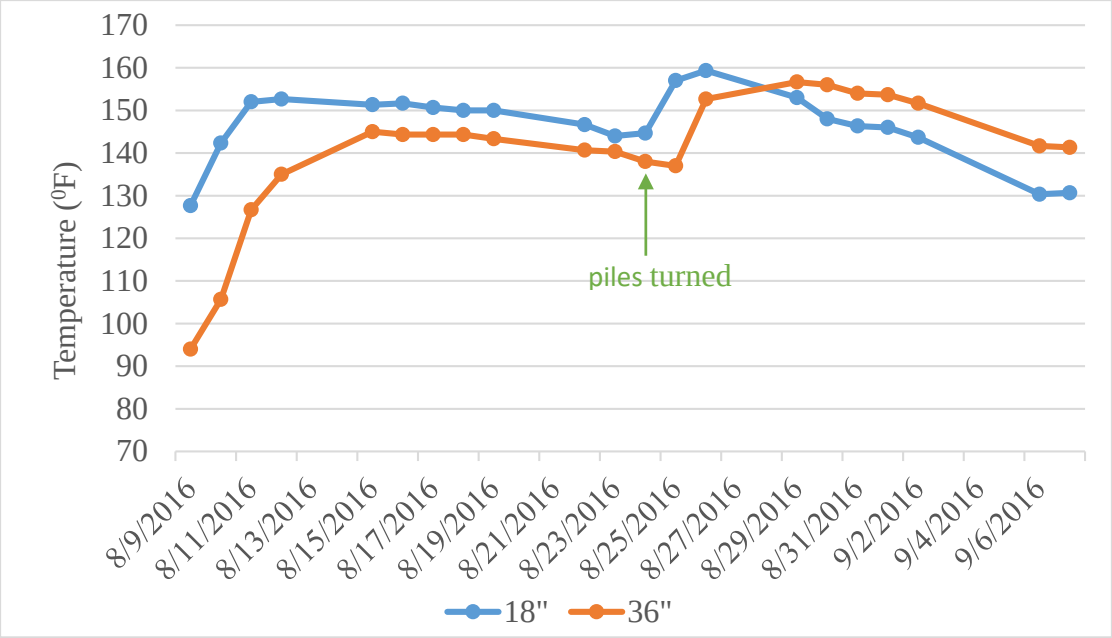

Figure 1. Average Temperatures for Horizontal Mixer Treatment

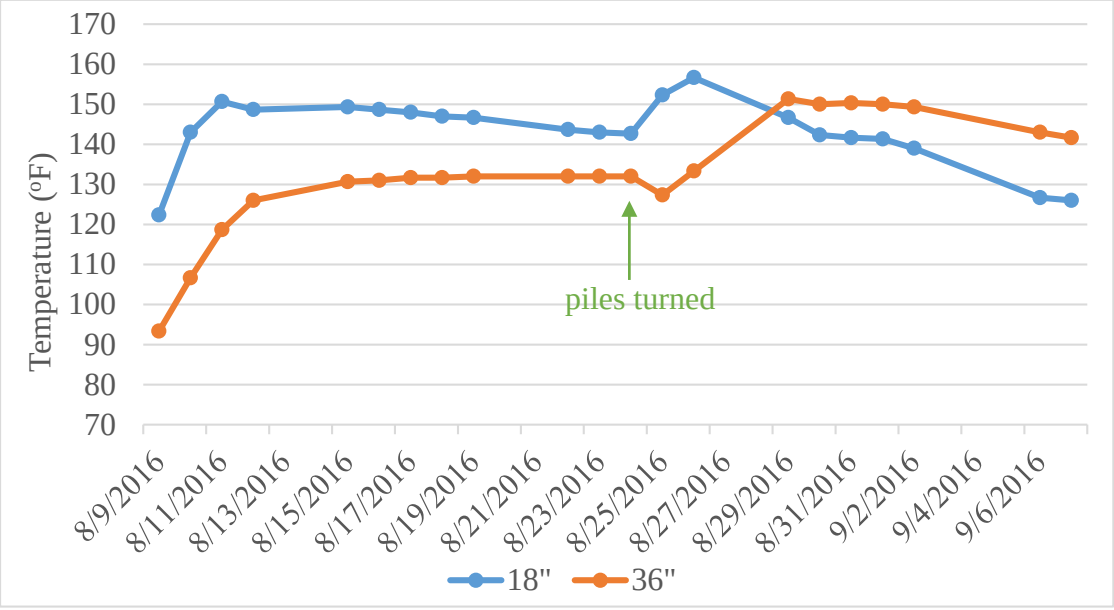

Figure 2. Average Temperatures for Conventional Layering Treatment

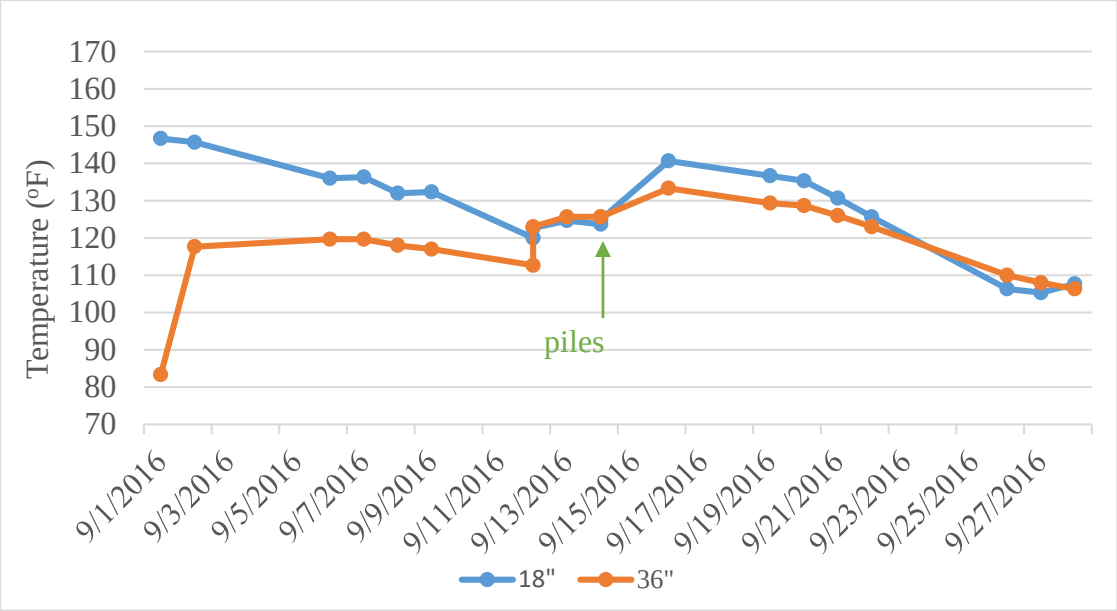

Figure 3. Average Temperatures for Vertical Mixer Wagon Treatment

Screen Weights

Screen weights for both the HM and VM treatments significantly decreased (p=0.09 and 0.00001 respectively) in the first 2-week cycle, whereas there was no significant difference in the CL treatment (Figure 4). This was due to the presence of whole birds on day 0 in the CL treatment, and the inability to get a true screen weight value with a small 2-gallon sample size. The 2-gallon sample either had a whole bird or no birds at all depending on where the pile was sampled. The screen weights were significantly higher for the VM treatment on day 0 than for the CL treatment (p=0.008). This is also explained by the presence of whole birds and dry carbon material in the CL treatment and an evenly distributed mixture of bird parts and carbon material in the VM treatment.

At the end of the first 2-week cycle, there was no significant difference between the screen weights for the HM (1.08 lbs.) and the CL (1.95 lbs.) treatments or between the VM (0.73 lbs.) and HM treatments. However, there was a slightly significant difference (p=0.10) between the VM and CL treatment screen weights. Screen weights on day 16 for the CL treatment had a

large variance due to the continued presence of whole birds in some samples and only dry carbon in other samples, in comparison to small variances for both the HM and VM treatments.

By the end of the composting trials, the final screen weights for the VM (0.67 lbs.) treatment were significantly lower than the screen weights for both the HM (1.21 lbs.) and CL (1.61 lbs.) treatments (p=0.05 and 0.01, respectively). There was no significant difference between the HM and the CL treatment screen weights. In the second 2-week cycle, there was no significant change in screen weights for the HM, CL, or VM treatments.

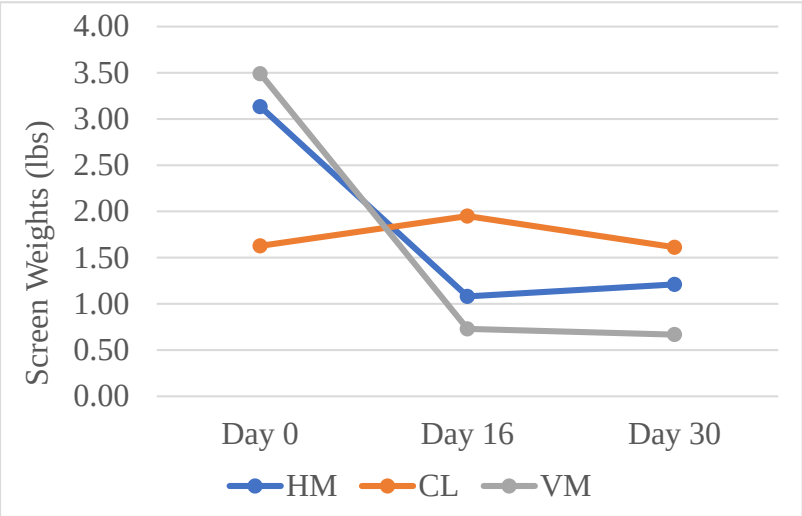

Figure 4. Average Screen Weights for CL, HM and VM Treatments Day 0, 16*, and 30*

*Actually Day 14 and 28 for VM treatment

Economic Calculations

Table 1 lists minimal times for the CL, HM, and VM treatments per 254 birds (the average number of birds per bucket load/pile). These times were used to calculate the number of hours it would take to process 200,000 birds based on the small-scale set up in our trials. When calculating the costs for the HM or VM treatment versus the CL treatment in Table 2, the size of equipment and quantity of equipment and operators was scaled up to process more birds in the same amount of time. It was approximated that if two 5 cubic yard loaders with 2

operators (instead of a ¾ cubic yard loader) and at least a 24-cubic yard mixer wagon (instead of a 15-16 cubic yard mixer) were used, 1700 laying hens with manure or 3400 laying hens without manure could be processed in the average 15 minutes.

While the Iowa HPAI outbreak farm interviewed for this study used a Tub grinder (rented at $500/hr including fuel and operator) and Tebbe manure spreaders (rented at $180/hr per spreader including fuel and operator), it was assumed that the mixer wagon rental rates would be similar to the Tebbe manure spreader and would take the place of both the tub grinder and manure spreader. This rate assumption seemed reasonable when a local Iowa equipment dealer gave a rental rate of approximately $80-100/hr for their vertical mixers without fuel and operator costs. The CL method for both layer and turkey farms required at least one additional laborer on the ground due to the tendency for whole birds to roll off the pile and necessitates more labor to replace them and more carbon material to cover them up (Table 2 and 3).

For a layer operation outbreak, when using at least a 24-cubic yard mixer and 2 five cubic yard loaders, 200,000 layer birds can be processed in 20 hours without manure and 30 hours with manure and costs between $15,000 and $37,256, depending on supply and demand and the amount of manure that needs to be composted. In comparison, the CL method takes approximately 35 hours to complete and costs between $20,500 and $48,300 depending on the supply and demand and the amount of manure per 200,000 birds (Table 2).

Discussion

Avian influenza outbreaks in the U.S. have become increasingly more common in the past couple of decades. Due to its severe impacts on food security, international trade, and human health, AI is an important disease that requires thorough surveillance as well as efficient and timely response to eradication. During the most recent outbreak of HPAI in the U.S. from 2014-2016, composting and burial were the most common methods of carcass disposal

(Johnson, Seeger and Marsh). Due to the size of the outbreak, disposal efforts were challenged by availability of equipment, labor, and carbon material (Johnson, Seeger and Marsh). Bottleneck effects drove up the cost of labor and supplies and, subsequently, the speed of clean-up efforts. As an epizootic disease, it is imperative that efforts are made to improve response and disposal in the future. It is also important that the disposal methods do not pose a risk to biosecurity and environmental pollution and are as economically efficient as possible. While burial may be a less technical and easier solution to carcass disposal than composting, it brings risk of environmental pollution and, if taken offsite, to biosecurity. Concerns about virus survival with burial of infected carcasses have also been raised (Graiver et al.). In contrast, on-site composting carries little risk to biosecurity, is efficient at viral inactivation, poses minimal risk to the environment, and creates a useful end-product that can be marketed and utilized. For these reasons, further efforts should be made to improve and reduce the cost of composting methods to encourage its use as the preferred method of carcass disposal during FAD outbreaks.

During the composting trials at the Compost Research and Education Center, the VM treatment had superior tissue breakdown than the CL or the HM treatment and accelerated tissue decomposition. However, the HM treatment had superior peak temperatures during the first 2-week cycle compared to both the VM and CL treatments. Both the VM and HM treatments had significant decreases in particle size from the start of the trial to the end of the first 2-week cycle. While the VM treatment did not perform as well for temperature, this can be explained by inadequate moisture content and poor windrow formation. Additionally, pile thermometers sunk too low in the pile and were reset on day 12 causing a significant increase in temperature at the 36” level. Higher peak temperatures for the first cycle could have been missed due to improper thermometer placement. One challenge with the VM was the discharge door was too low to create a sufficiently high enough pile. This can be remedied with the

addition of a belt driven chute that can discharge material up to 6 feet tall. Alternatively, a loader could be used to push the piles higher. Despite these challenges, tissue break down was superior. While temperatures for this method did not meet the 131^{o} F for 3 consecutive days' standard set by the USDA Composting Protocol, it did meet 110^{o} F for 10 consecutive days for both cycles and temperatures were more than adequate to kill the virus. Other factors besides heat during composting have been shown to be important in virus inactivation as well (Glanville et al.; Guan et al.). The temperatures in all treatments in this study were consistently adequate for efficient virus inactivation within compost piles according to published research (Lu et al.; Chumpolbanchorn et al.; Senne, Panigrahy and Morgan).

The VM and HM treatments also support shortening of the current requirement of a 4-week composting cycle. The data from the HM and especially the VM treatment showed a significant difference in particle size in the first 2 weeks, but no difference in the second 2-week cycle. Since virus inactivation has been demonstrated to occur within 24 hours at temperatures as low as 25^{o} C (77^{o} F) in manure (Chumpolbanchorn et al.) and as low as 42^{o} C (107^{o} F) in compost (Senne, Panigrahy and Morgan), a 2 week cycle with turning of the piles after 7 days to ensure homogeneous temperatures and mixing, should be ample time to achieve neuralization of the virus. While shortening of the composting cycle may not have direct effects on the cost of carcass disposal, it could have an immense impact on reducing the opportunity costs to producers if their barns or fields were only occupied for half the time. This could accelerate the cleaning and disinfecting of the barns, allowing producers to restock their flocks sooner and encourage producers to choose composting over burial. While the compost product would not be ready for field application as a soil amendment, it could be safely moved to an approved storage site without biosecurity risk.

Even without the reduction in opportunity cost with a shorter composting cycle, the economic calculations in this project support the use of a mixer wagon for carcass composting.

The reduced amount of carbon material and manual labor required, as well as the increased speed with larger equipment, make it an economical choice. A similar method of particle reduction size and windrow construction was achieved during an outbreak in Iowa and not only proved to be effective but also economical (Elbert). Other forms of equipment, such as the tub grinder and Tebbe manure spreaders used in Iowa, could be considered, depending on availability and cost. In large agricultural regions, such as Iowa, equipment such as feed mixers may be more available than in other regions of the country.

During the 2014-2016 AI outbreak, reports of prolonged times between depopulation and carcass disposal have been reported due to shortages in labor, equipment, and carbon sources during the height of the outbreak (Johnson; Elbert). Several lessons can be learned from this, including the importance of securing equipment, labor, carbon sources, and other supplies for each state prior to an outbreak. These preparations not only help insure adequate resources are available and efficiently acquired but can also help reduce hikes in cost if contracts are already in place. Reduced costs could make composting even more economically feasible and reduce the amount of facilities that choose burial as their method of disposal. Poultry producers should be encouraged to develop emergency plans of their own as well.

The 2016 USDA APHIS protocol for carcass composting of AI flocks does not currently support the use of mixing or grinding equipment for carcass disposal due to the potential risk of virus aerosolization. This project did not address aerosolization concerns with the VM or HM and should be investigated in future studies. Additionally, due to the small sample size of this study, a larger and blinded trial could provide more information. A larger trial with a vertical mixer wagon with a 6-foot discharge chute and adequate moisture could improve the temperature profile and efficiency and provide more support for its use during an outbreak.

Conclusion

Particle size reduction and mixing improves and accelerates degradation rates of poultry carcasses in compost. This study indicates that a vertical or horizontal mixer wagon is an economical method for processing poultry carcasses. Other equipment that achieves particle size reduction and mixing should be considered depending on equipment availability in different regions of the country. Besides a reduction in the direct costs of carcass composting with efficient handling of carcasses, manure, and carbon material, reduced opportunity costs for producers could be achieved with a shorter composting cycle based on current research on AI virus stability in compost and manure. Direct cost reductions for composting could also be achieved if states and producers planned for equipment, labor, carbon material, and other supplies prior to an outbreak. These reductions in direct and opportunity costs could encourage more producers to select composting as the means for carcass disposal over burial, which can have additional costs associated with groundwater contamination and prolonged virus inactivation as well as reduced value of property.

Table 1. Minimal Processing Times for HM, CL, and VM Treatments with 1 (¾ yd^3) Loader, 1 Tractor Operator, 1 Mixer and 1 Laborer, with Manure

CL	VM (16.3 yd^3)	HM (15.5 yd^3)
14 min per 254 birds	16.22 min per 254 birds	14.17 min per 254 birds
11,023 min per 200,000 birds	12,600 min per 200,000 birds	11,157 min per 200,000 birds
184 hours per 200,000 birds	210 hours per 200,000 birds	186 hours per 200,000 birds

Table 2. Economic Estimates for Conventional Layering and Mixer Wagon Methods for Layer Hens

	COST PER 200,000 birds			
MIXER WAGON METHOD	**No manure**		**With manure**	
2 days without manure, 3 days with manure (10hrs/day) per 200,000 birds	**Low**	**High***	**Low**	**High***
Equipment (fuel, equipment, operator included)				
5 yd^3 wheel loader making base/ cap ($125/hr)	$2,500	$3,125	$3,750	$4,688
5 yd^3 wheel loader adding birds, carbon, manure to mixer ($125/hr)	$2,500	$3,125	$3,750	$4,688
1 tractor with mixer ($180/hr)	$3,600	$4,500	$5,400	$6,750
Labor				
1 Laborer on the ground ($20/hr)	$400	$500	$600	$750
1 Foreman ($40/hr)	$800	$1,000	$1,200	$1,500
Carbon Material				
600 -1180 cubic yards ($9-16 /$yd^3$)	$5,400	$9,600	$10,620	$18,880
Total cost	**$15,200**	**$21,850**	**$25,320**	**$37,256**
CONVENTIONAL LAYERING METHOD				
3.5 days (10hrs/day) per 200,000 birds	**Low**	**High***	**Low**	**High***
Equipment (fuel, equipment, operator included)				
1 Track skid loader making base/ cap ($100/hr)	$3,500	$4,375	$3,500	$4,375
1 Track skid loader layering carbon/litter ($100/hr)	$3,500	$4,375	$3,500	$4,375
1 Track skid loader layering birds ($100/hr)	$3,500	$4,375	$3,500	$4,375
1 Track skid loader layering manure ($100/hr)			$3,500	$4,375
Labor				
2 Laborers on the ground ($20/hr)	$800	$1,000	$800	$1,000
1 Foreman ($40/hr)	$800	$1,000	$800	$1,000
Carbon Material				
900 -1800 cu yd ($9-16/ yd^3)	$8,100	$14,400	$16,200	$28,800
Total cost	**$20,200**	**$29,525**	**$31,800**	**$48,300**

Based on recorded times and carbon amounts for each method at Highmoor Farm, costs from HPAI outbreak farm in Iowa in 2015 (7) and carbon amounts and costs from HPAI influenza outbreak farms (10)

*High estimate is 1.25 times the low end to allow for changes in supply and demand

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CO2 Culling with Influenza Containment System I.C.S.: Physiological and Ethical Considerations

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June 2018 | Amarillo, Texas

CO_2 CULLING WITH INFLUENZA CONTAINMENT SYSTEM (I.C.S.): PHYSIOLOGICAL AND ETHICAL CONSIDERATIONS

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Summary

This study evaluates the use of the I.C.S.-bag compared with current killing methods for poultry. The ICS culling method was evaluated from several ethical standpoints with the 'Animal Disease Intervention Matrix' (ADIM, Aerts 2006). This system provides governments with a tool to take more ethically justified decisions about animal diseases. In a series of gassing experiments on laboratory scale, the changes in the physiological mechanisms and the behavioural changes of birds after exposure to rising CO_2 were investigated. Finally, it was determined if the I.C.S.-bag was bio-secure for Highly Pathogenic Avian Influenza (HPAI) over a period of 48 hours. This study was performed by the Instituto Zooprofilattico delle Venezie (IZSVe, Italy).
Finally, this system was introduced at the University of Lomé, Togo. There the method was used during one of the latest out-brakes. Also, based on the ability of the system to contain HPAI and no leakage when gases are injected into the system, storages tests were conducted on poultry feed and hatching eggs. The results showed that the I.C.S.-bag has (1) a higher ADIM-score, (2) death within 40 seconds and (3) no dispersion of the virus in the environment within 48 hours. The other experiments indicated (4) a higher Feed Conversion Ratio when stored in the I.C.S.-bag, as well as (5) higher hatching rate of day old chickens.

Introduction

Avian influenza Directive 2005/94/EC of the Council of 20 December 2005, which was incorporated into national legislation at the end of 2006, differentiates between high pathogenic (HPAI) and low pathogenic (LPAI) avian influenza. Low pathogenic avian influenza does not make the animals ill, but according to scientists, it may mutate to high pathogenic avian influenza, which can make chickens seriously ill and may infect humans. In both cases the poultry at the infected site and its surroundings must be evacuated. As a pest control measure, all infected animals and those within a certain radius of the infected location are killed. This applies to poultry kept by both amateurs and professionals.

The most commonly used procedures for large-scale emergency depopulation of birds, e.g. in the case of avian influenza, consist of exposing poultry to gasses. Many different gas types and mixtures are used for stunning and killing poultry. With the exception of CO and HCN killing, gas mixtures contain three important components: CO_2, N_2 and Ar. Gas killing can be done without removing the animals from their housing ('whole house gassing'), in an environment containing at least 45% CO_2 for killing all animals (Gerritzen et al., 2006; Raj et al., 2006; OIE guidelines, 2005).

From the point of view of both human and animal welfare, the killing of animals in the shed with a minimum of man-animal interaction is preferred. Hitherto, the killing of poultry in the shed has only been possible by means of shed gassing with CO_2. It is not possible to use this method in every shed because - sheds with excessive leakage cannot be effectively responsibly filled with CO_2 gas.

Recently, a new gassing system was developed in which gassing is performed in an I.C.S.-bag filled with CO_2 gas or dry ice. However, it was not clear how the birds would react and how blood acid-base related parameters change when birds are exposed, immediately or gradually, to an environment with very high CO_2 / very low O_2 concentrations. Moreover, information was lacking on spatial and time distribution of CO2 in an I.C.S.-bag and in the close surroundings of the bag, since safety of the workers has to be guaranteed. These issues were clarified in several of our experiments.

Materials and Methods

A group of 6-week old broilers was divided into three groups and the broilers were individually killed by direct exposure to high CO2 (approx. 57% CO2) in the I.C.S.-bag (exp 1), by means of gradual and rapid build-up of CO2 in a plexibox (exp 2) and by means of gradual and slow build-up of CO2 in a plexibox (exp 3). Venous blood samples were taken from the animals' wing vein before and after gassing and immediately analysed using a blood gas analyser. These samples were used to measure the parameters relating to the acid-base control in the blood (blood pH, partial pressure of CO_2, partial pressure of O_2, HCO_3^-, base excess, haematocrit, acid saturation, Na^+, K^+, Ca^{2+}) by means of a blood gas analyser (pGEM3000, Instrumentation Laboratories). In addition, glucose and lactate were measured.

The time of death was diagnosed by measurement of heartbeat, respiration and corneal enlargement. The behaviour of the animals was observed and the time of behaviour-related change, as defined by Gerritzen et al. (2004), was observed and noted. The time and occurrence of the different behaviours during the CO_2 stunning were recorded with a PC-based data-acquisition system (National Instruments).

Post-mortem muscular rigidity was checked using the PUFF system (Bamelis et al., 2006) as an indicator of post-mortem meat quality. The PUFF control animals were four additional broilers that were killed by cervical dislocation.

An O_2-sensor (Vaisala OMT355) was chosen to measure the changes of CO_2, instead of a CO_2-sensor. The reason was that the O_2-sensor was more accurate and reliable than a CO_2-sensor. Since all experiments were performed under normal atmospheric conditions, it may be assumed that a constant ratio subsists between the % of O_2 measured and the % of CO_2. The ratio was calculated as follows: Assuming a standard air mixture consisting of 20.94 % O_2, 0,03 % CO_2 and residual gases (N_2, Ar, …): "the % CO_2 after the addition of CO_2" = 100(1-('%O_2'/20.94)).

Results

Experiment 1. Immediately after being placed in the I.C.S.-Bag, all the animals began wing flapping and showed uncontrolled behaviour. Death of all the animals was diagnosed within 40 seconds (Table 1). Venous blood values pointed to significant acidification of the blood (fall of pH – Table 2). Nevertheless, no other blood parameters changed significantly through direct exposure to high CO_2 concentrations, except Na^+ and Ca^{++}. Most probably, the time of exposure was too short to produce any significant changes in the venous blood.

Table 1. Time of reaching well-defined behaviour on exposure to a rapidly rising concentration of CO_2. The times are shown as an average ± standard deviation. Likewise given are the O_2 and CO_2 concentrations at which a well-defined behaviour-related change occurs.

	Deep respiration with gasping and neck stretching	**Loss of posture**	**Wing flapping and uncontrolled muscular movement ('convulsions')**	**Loss of movement ('motionless')**
Experiment 1 Very rapid gassing in I.C.S.-Bag (n=6)	/	/	/	<= 40 seconds
Experiment 2 Rapid gassing in a plexibox (n=7)	12 ± 3 seconds	49 ± 12 seconds	59 ± 11 seconds	106 ± 15 seconds
	O_2: 19.6 ± 0.1 %	12.5 ± 0.2	11.1 ± 0.1	
	CO_2: 6.2 ± 0.4 %	40.1 ± 0.8	46.8 ± 0.7	6.9 ± 0.2
				66.7 ± 0.9
Experiment 3 Slow gassing in a plexibox (n=3)	227 ± 100 seconds	420 ± 176 seconds	900 ± 101 seconds	1209 ± 345 seconds
	O_2: 18.8 ± 0.5%	17.7 ±0.4 %	16.0 ± 0.7 %	14.2 ± 0.2 %
	CO_2: 10.1 ± 2.5%	15.7 ± 2.1 %	23.6 ± 3.2 %	32.1 ± 0.9 %
	Gerritzen (2004): 46 seconds CO_2: 6 %	Gerritzen (2004): 172 seconds CO_2: 15.7 %	Gerritzen (2004): 177 seconds CO_2: 16 %	Gerritzen (2004): 700 seconds CO_2: 31.5 %

Table 2. Blood parameters of broilers gassed with CO2 in different circumstances

	Experiment 1 Very rapid gassing in I.C.S.-Bag (n=6)		**Experiment 2 Rapid gassing in a plexibox (n=7) ***		**Experiment 3 Slow gassing in a plexibox (n=3) ****	
	before	**after**	**before**	**after**	**before**	**After**
pH	7.34 ± 0.02[a]	7.24 ± 0.03[b]	7.39 ± 0.04[a]	7.24 ± 0.03[b]	7.35 ± 0 .01	6.86 ± 0.06
pCO_2 (mm Hg)	37.5 ± 3.0	37.5 ± 2.2	51.8 ± 7.0	62.2 ± 6.3	42.5 ± 4.5	99.5 ± 5.5
pO_2 (mm Hg)	53.8 ± 3.7	47.7 ± 4.8	47.0 ± 1.6[a]	30.2 ± 2.8[b]	50.5 ± 1.5	31 ± 2.0
SO_2 (%)	83.7 ± 3.8	65.2 ± 9.4	81.5 ± 2.6[a]	45.2 ± 7.0[b]	83 ± 1.0	22.0 ± 0.0
Glucose (mg/dl)	207.3 ± 10.5	186.8 ± 14.4	202.3 ± 7.5	181.0 ± 8.0	211.5 ± 5.5	181.5 ± 30.5
Lactate (mmol/l)	4.4 ± 0.5	5.7 ± 0.5	4.3 ± 0.8	7.0 ± 0.6	4.2 ± 0.3	11.4 ± 2.6
Haemato-crit	25.5 ± 0.7	23.8 ± 1.8	30.2 ± 1.3	33.8 ± 0.7	26.5 ± 2.5	25.0 ± 4.0
HCO_3^- (mmol/l)	20.3 ± 1.6	16.5 ± 1.6	30.7 ± 2.3	25.5 ± 1.6	23.7± 1.7	17.8 ± 1.5
Base excess (blood) (mmol/l)	-4.9 ± 1.5	-9.9 ± 1.8	5.66 ± 1.8	-5.3 ± 3.9	-1.7 ± 1.3	-15.3 ± 2.9
Na^+ (mmol/l)	132.5 ± 2.9[a]	118.5 ± 4.1[b]	147.8 ± 1.8	151 ± 1.0	133.5 ± 0.5	129.5 ± 2.5
K^+ (mmol/l)	5.23 ± 0.1	5.73 ± 0.17	/	/	5.3 ± 0.3	5.7 ± 0.2
Ca^{2+} (mmol/l)	1.22 ± 0.05[a]	0.99 ± 0.07[b]	1.39 ± 0.03	1.36 ± 0.03	1.23 ± 0.02	1.29 ± 0.01

* Statistical analysis is carried out by means of repeated measurements (SAS); various letters point to significant differences of a particular parameter before and after gassing.

** in view of the small number of observations, no statistical analysis of experiment 3 data was carried out

Experiment 2. A typical progress of the O_2 concentration and related CO_2 concentration, during the gradual build-up (rapid) is shown in Figure 1. This figure shows also the changes in the heartbeat rhythm. The duration of behaviour related modification is shown in Table 1. The variation between animals was not great, pointing to the same behaviour pattern of the animals' reaction to high CO_2 concentrations. The sequence of the occurrence of these behaviour-related changes was the same in all the animals. Following the diagnosis of death, all animals were lying on their backs. Table 2 shows the blood parameters of the animals before and after gassing. Blood pH fell significantly, coupled with an insignificant rise of partial pressure of CO_2 and a fall in HCO_3^-. Base excess fell significantly as a result of exposure to CO_2. The partial pressure of O_2 and oxygen saturation of venous blood, were significantly lower after gassing. There was a tendency towards a high lactate and a lower glucose concentration after gassing, electrolytes were not changed. The pattern of progress of *rigor mortis* observed using the PUFF system did not differ between CO_2-gassed animals and animals killed by cervical dislocation (results not shown).

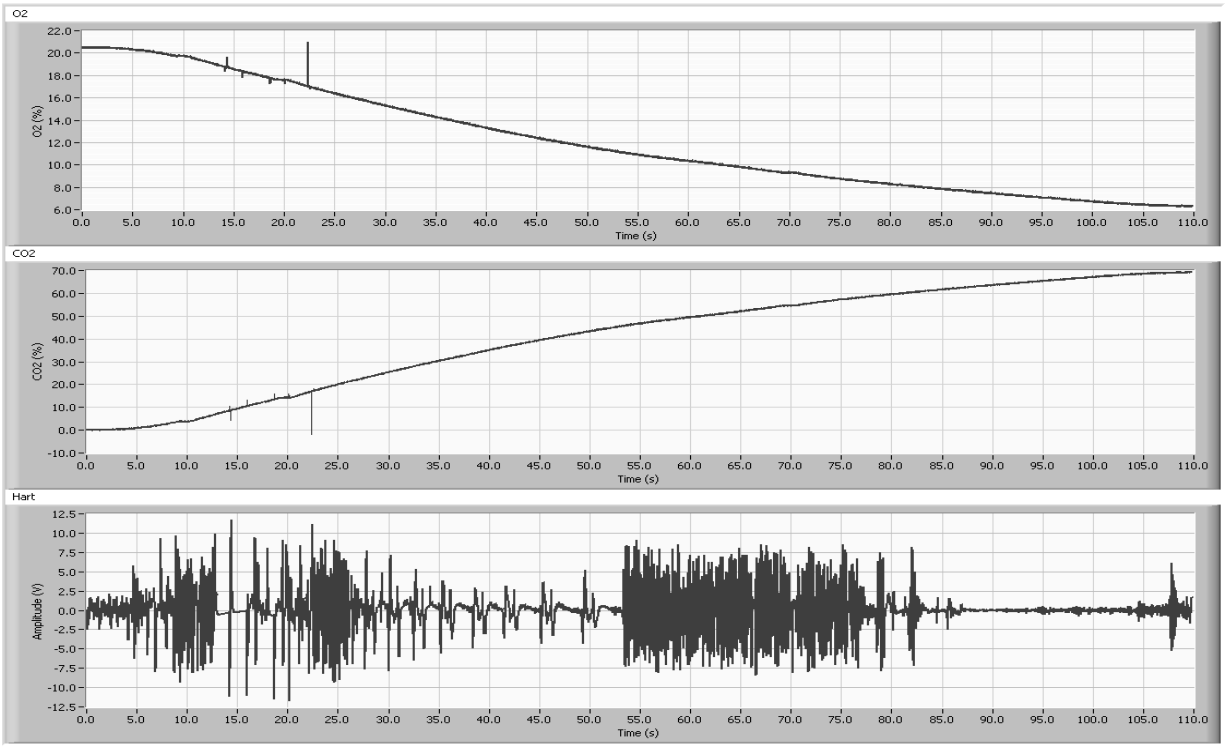

Figure 1. Progress of O_2 and CO concentrations and of heartbeat during rapid gassing.

Experiment 3. A typical progress of the O_2 concentration during slow gassing with CO_2 is shown in Figure 2. Since the build-up of CO_2 occurred very slowly, the timing of the sequence of the different behaviour patterns was also appreciably delayed (Table 2), with occurrence of death after only 20 minutes. Likewise note the great similarity in CO_2 concentrations to the results of Gerritzen et al. (2004), although the timing in our experiment was different. Marked differences can also be noted in O_2 and CO_2 concentrations, respectively in experiments 2 (rapid) and 3 (slow). All animals died lying on their stomachs. The blood parameters of the animals are shown in Table 2. There was a marked fall of blood pH occurred coupled with a markedly higher partial pressure of CO_2 and lactate concentration. Base excess and bicarbonate concentrations fell strongly. The

partial pressure of O_2 underwent a fall and oxygen saturation was strongly reduced after gassing. Haematocrit and electrolytes remained almost unchanged.

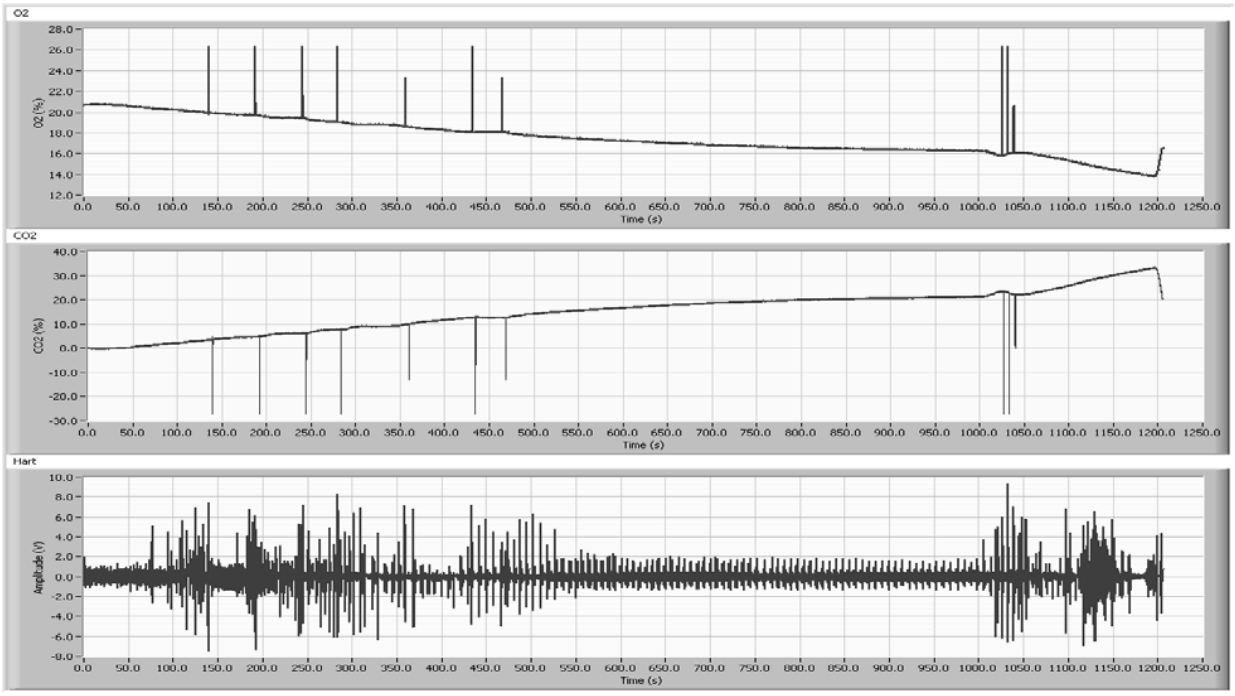

Figure 2. Progress of O_2 and CO_2 concentrations and of heartbeat during slow gassing.

Discussion

It is clear from the observations that ***CO_2 gassing in the I.C.S.-Bag*** (experiment 1) resulted in very rapid death (within 40 seconds). This rapid death was probably attributable to a number of causes. Both an acute exposure to a very low O_2 concentration (9%) and to a very high CO_2 concentration (calculated: 57%) caused specific reactions in the animal.

The results of Zeller et al. (1988) showed that in the case of an acute exposure to a high CO_2 concentration (50% for 30 seconds), an immediate rise of pCO_2 occurred (from 20 mmHg to 120 mmHg) in the arterial blood of cannulated broilers. This was coupled with an immediate fall of blood pH (from 7.5 to 6.9 in 30 seconds), which was also to be observed in our experiment, although the fall was less pronounced. There was no pronounced fall of the partial pressure of O_2 within 30 seconds in arterial blood (Zeller et al., 1988), or in venous blood in our experiment.

Exposure to 40% CO_2 for 60 seconds caused a rapid fall of both systolic and diastolic blood pressure, together with a heart arrhythmia and marked bradycardia (slow heartbeat). These data of Zeller point to the fact that acute exposure to very high CO_2 concentrations; resulted into rapid changes of acid-base parameters, but not of partial pressure of O_2 blood values neither in arterial nor venous blood, at least over a very short space of time. In the case of inhalation of very high CO_2 concentrations, breathing stops immediately (Zeller et al., 1988). In contrast with mammals, birds possess intrapulmonary chemoreceptor's that are acutely sensitive to CO_2, but insensitive to hypoxia or anoxia (Ludders, 2001), stimulation of the said receptors leads to a suppression of

breathing via the vagus nerve. The extent and speed of the inhibition depends on the inhaled CO_2 concentration or the blood partial pressure of CO_2.

Zeller et al. (1988) also described a rapid fall of blood pressure and bradycardia which point to a direct toxic effect of CO_2 on heart function; this can be related to the flapping around of the animals, comparable with the situation of the so-called flip over of broilers (indication of heart failure). These immediate effects of direct exposure to very high CO_2 concentrations explains why no significant rise occurred in venous blood partial pressure of CO_2, because gas exchange stopped almost immediately. There was a trend to a lower degree of oxygen saturation in venous blood; this change was probably more pronounced in arterial blood. Although the trends were present, the duration of time during which the animals were killed was probably too short to observe other significant blood changes in relation to the acid-base balance.

Gassing in a plexibox (experiment 2), characterised by exposure to ***gradually rising CO_2 and falling O_2 concentrations***, caused a mild metabolic acidosis in the animals (by definition a primary HCO_3^- reduction coupled with a fall of pH) in venous blood. Under this condition the animal will try to compensate for this metabolic acidosis by an increase in the volume of breath per minute. However, owing to the high concentration of CO_2 in the inhaled air, the partial pressure of CO_2 will rise dramatically, whereby breathing will be suppressed more rapidly. This was reflected in the deep slow breathing of the animals in this experiment.

The rapid fall in the blood pH observed in our experiment caused a fall in pH of the cerebrospinal fluid and intracellular in the brain cells, as previously reported in dogs and pigs (Eisele, 1967; Martoft et al., 2003). Blood pH and the cerebrospinal fluid pH moreover appear to be strongly correlated. The said fall of pH has been shown to cause anaesthetic effect, since CO_2 is capable of suppressing nerve cell function and cerebral electrical activity (Eisele, 1967). Attaining this pH threshold value that causes anaesthetic effect may coincide with the loss of balance and the closing of the animal's eyes (after 50 seconds); this type of behaviour will indicate a loss of consciousness (Raj et al., 1998).

As well as the said acidosis, there was also a clear effect of associated *hypoxia*, which translated into a significant fall in partial pressure of O_2 and oxygen saturation. Hypoxia normally causes a rising stroke volume and heartbeat. In terms of behaviour however, no increase in the speed of breathing could be observed, which may point to interference with the consequences of the high CO_2 concentrations. Presumably, the hypoxia only increased after the loss of consciousness and this occurred only at the end of bradycardia and arrhythmia. This finally led to heart failure and the animals died on their backs in experiments 1 and 2 wich is another indication of heart failure.

Animals that were subjected to gradually increase in CO_2 concentration continued to breathe for a time, causing a significant fall in partial pressure of O_2 due to a prolonged exposure to low O_2 concentrations. Animals that were immediately exposed to very high concentration of CO_2 / low concentration of O_2 presumably suffered immediate breathing arrest and heart failure. Similar blood changes were noted in turkeys and ducks (Gerritzen et al., 2006), although absolute blood values differed strongly from those of broilers as higher CO_2 concentrations were needed to reach a loss of consciousness.

Typical consequence of rapid gassing (experiment 2) was the rapid sequence of behaviour-related changes that can be linked to the attainment of a clearly defined O_2/CO_2 concentration. A similar sequence of behaviour-related changes was described by Webster and Fletcher (2001) and by Gerritzen et al. (2004), although the timing of the occurrence of this behaviour differed appreciably from that of slow CO_2 build-up (Gerritzen et al., 2004). In addition, we were able to establish clearly in our study that the occurrence of the loss of balance, which may be a first indication of loss of consciousness, preceded the phase of convulsions. This could not be established in the large test group used by Gerritzen et al. (2004).

Slow gassing (experiment 3) showed the same sequence of behaviour-related changes over a longer period, but with the difference that the animals died slowly lying on their stomachs. The loss of consciousness occurred after approximately 420 seconds at an 0_2 concentration of 17.7% and a CO_2 concentration of 15.7%, exactly the same CO_2 concentration which Gerritzen et al. (2004) quoted for reaching loss of consciousness. In the case of shed gassing, the attainment of 40-45% CO_2 concentration and its maintenance for 30 minutes has been quoted as adequate for killing animals (Gerritzen et al. 2006). This corresponds to 12.6 – 11.5% O_2. In our experiment the animals died on their stomachs after a gradual build-up of CO_2 to a final concentration of 32.1% (O_2 14.2 %) after approximately 20 minutes.

The slow CO_2 gassing resulted in very pronounced *metabolic acidosis*, which was characterised by a fall of pH and also by strong falls of the buffering systems (bicarbonate and non-bicarbonate buffer bases). In addition, there was a strongly increase in lactate concentration in the blood. The very low level of oxygen saturation indicated a *severe deficit of oxygen*.

Acknowledgement

This research was funded by the Institute for the Promotion of Scientific and Technological Research in Flanders (I.W.T. Vlaanderen) in the framework of the project IWT070324.

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