RESEARCH REPORT
SWINE HEALTH

Title: Virus Survival in Preprocessed Compost Under Cold Conditions - NPB #20-016

Investigator: Brent Pepin

Institution: Pipestone Veterinary Services

Date Submitted: 6/12/2020

Industry Summary:
The objectives focused on assessing the usability of preprocessing (grinding) carcasses before composting for disposal after a break of foreign animal diseases like the African Swine Fever Virus (ASFV). The primary study objectives included:

1. Assess if the preprocessing (grinding) of carcasses for compost is a risk for aerosolization of swine viral pathogens
2. Determine if preprocessing and composting of swine carcasses under cold weather conditions (winter in the Midwest United States) will adequately kill swine viral pathogens (PRRSV and PEDV)
3. Measure temperature readings to ensure preprocessed compost material reaches critical temperatures under winter conditions to kill swine pathogens beyond the surrogates used in this study (foreign animal viruses like ASFV)
4. Analyze groundwater samples for potential leaching of swine viral pathogens from the composting material

The study was conducted in February in Minnesota to recreate a scenario of mass carcass disposal from a depopulation event in cold weather. A horizontal grinder designed for grinding lumber was used to preprocess the carcasses before forming compost windrows. Three different compost biomass types were used, including woodchips, cornstalks, and a 1:1 ratio of woodchips/cornstalks. Each biomass type was preprocessed separately with carcasses and made into their own compost windrow (3 windrows total). Surrogate disease positive pigs (exposed to PRRSV and PEDV) were used to analyze the detection and degradation of swine viral pathogens overtime in the compost windrows. Air samples were collected during the carcass preprocessing, with the air collector set up downwind from the horizontal grinder to monitor for potential aerosolization of viruses. Water wells were placed at three different depths (6”, 18”, and 36”) under each biomass type and 25 feet downhill from the windrows. Daily for the first 5 days and then weekly, temperature readings and two compost samples were collected for PCR testing from each windrow. Water well collection attempts were made once weekly until the end of the study from each well. Compost and water well samples were all tested by PCR for PRRSV and PEDV.

The results of this study revealed that preprocessing of swine carcasses before composting reaches temperatures expected to kill Foreign Animal Diseases like ASFV, even under cold weather conditions (winter conditions in the upper Midwest United States). Each compost biomass type reached temperatures ≥140°F for multiple consecutive days, which is the necessary temperature

required to kill off ASFV when held for 15-20minutes. The air samples collected during the preprocessing and the weekly water well samples revealed the risk of environmental contamination and virial leaching to be minimal under the study conditions. The PCR testing of the compost samples revealed that woodchips appear to harbor PRRSV and PEDV nucleic acid longer than cornstalks (by themselves and in combination). However, after two weeks, each biomass type tested negative for both PRRSV and PEDV nucleic acid.

Overall, the results of the current study confirm that the preprocessing and composting method of infected swine carcasses is a potentially viable method for mass disposal in events such as a foreign animal disease break. Further benefits of preprocessing carcasses include decreasing the required land space and carbon material needed for the composting process. Knowing biomass materials other than woodchips that can adequately reach pathogen eliminating temperatures is beneficial as woodchips may be challenging to come by in some regions of the country or during periods of high demand. To quickly eradicate a disease like ASFV, a disposal method that does not promote the further spread or harboring of pathogens is required. The current study reveals that the preprocessing of carcasses and composting is of low risk for environmental contamination and spread, under the conditions of the study. Little information is available on the survivability of common swine pathogens in compost in outdoor conditions (most studies performed indoors and in controlled weather conditions). This study confirms that composting preprocessed carcasses can eliminate common swine pathogens and expected to do the same for foreign animal pathogens like ASFV.

For questions or further information, please contact:
Brent Pepin DVM, MS
Brent.pepin@pipestone.com

Key Findings:

- Even in cold weather conditions – preprocessing carcasses for compost achieved the required temperature expected to kill ASFV and common swine pathogens
- Risk of aerosolization of swine pathogens during carcass preprocessing appears minimal
- Leaching of viruses from compost windrows into groundwater appear low risk
- Duration of detection of virus nucleic acid in compost differs between biomass types

Keywords: Grinding, compost, ASFV, PRRSV, PEDV, preprocessing

Scientific Abstract:

The elimination of a foreign animal disease like the African Swine Fever virus (ASFV) requires an efficient means of disposal for infected or exposed animal carcasses. The disposal method must prevent the further spread of disease and reliably kill or inactivate infectious viruses. Limited studies have been performed on the monitoring of swine viruses over time in compost piles and studies on the preprocessing of carcasses before composting is based primarily on cattle carcasses. With the majority of the pig population in the United States residing in the Midwest, cold winter conditions are a concern for the proper execution of the composting process. Most compost studies in the literature are performed under very controlled environments, and not the adverse weather conditions where most composting occurs. Therefore, this study aimed to evaluate the ability to preprocess of swine carcasses for compost to eliminate viral pathogens in the face of adverse weather conditions (winter the Midwestern United States). This study further evaluated the preprocessing method for the potential risk of environmental contamination via aerosolization as it has not been previously addressed. The risk of leaching of pathogens into groundwater was also analyzed to ensure further viral spread is limited from the composting process. Carcasses of pigs exposed to Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Porcine Epidemic Diarrhea Virus (PEDV) were used as virus surrogates for testing and monitoring over time. Aerosol

sampling during the carcass preprocessing was performed. Water wells at various depths were placed for weekly monitoring of groundwater. Three compost biomass materials (woodchips, cornstalks, and 1:1 ratio of both) were used to form separate compost windrows of each type for comparison of ability to eliminate virus over time and for the ability to reach appropriate composting temperatures. Reverse transcription real-time polymerase chain reaction (PCR) testing of air samples, groundwater, and compost composite samples for PRRSV, and PEDV were performed. An on-site weather station monitored weather conditions (daily high and low temperatures and wind speed). The results of the study revealed that the composting of preprocessed carcasses was able to reach adequate temperatures for the elimination of ASFV and common swine virial pathogens, even in cold weather conditions. Aerosol and groundwater contamination from carcass preprocessing and composting appears minimal under the conditions of the study. Although each biomass windrow type was effective at reaching adequate temperatures, there was a difference in time until negative in surrogate virus detection. In conclusion, the preprocessing and compost method for carcass disposal is a viable potential method for pathogen control and elimination for ASFV and common swine pathogens.

Introduction:

African Swine Fever Virus (ASFV) is a highly contagious pathogen causing hemorrhages, high fever, and mortality rates approaching 100% (Ge et al., 2018). An introduction of ASFV into the United States (US) has a projected loss of $15 billion of revenue for the US pork industry in the first two years and up to $50 billion over ten years (Carriquiry et al., 2020). Methods for quick containment and disposal of infected or exposed animals are needed to keep potential financial loss to a minimum. The disposal of potentially infectious carcasses of exposed or infected animals requires a system that has minimal risk for further spread of disease and is effective at virus elimination. The Foot and Mouth Disease (FMD) elimination in the United Kingdom revealed the limitations of commonly used carcass disposal methods for mass disposals (Scudamore et al., 2002; Wilkinson, 2007). On-farm mass burial is restrictive due to risk to groundwater, burning has human safety and public perception concerns, and the use of rendering facilities and landfills require hauling of infectious carcasses off-site, which increases the risk of FAD spread (Scudamore et al., 2002; Wilkinson, 2007). However, composting is recognized as an environmentally friendly carcass disposal method in Australia, New Zealand, US, and Canada and can be completed on-site (Guan et al., 2010; Wilkinson, 2007).

Limited available studies have been completed on the use of compost for control of even common swine diseases like PRRSV and PEDV. Traditional composting (covering of full-body carcasses in carbon source biomass) is commonplace for regular daily mortalities. However, preprocessing of carcasses before composting may be more applicable for mass carcass disposal situations (as from depopulation events). Preprocessing of carcasses for compost requires less carbon biomass, less land space, and typically completes compost cycles faster than traditional compost methods (Erickson et al., 2004; Kalbasi-Ashtari et al., 2005; Rynk, 2003). The downside is most of the studies on preprocessing have been completed in cattle with limited information in swine and no tracking of virial elimination over time (Erickson et al., 2004; Rynk, 2003). Studies on composting of swine carcasses that look at virus elimination are typically performed indoors or inside containers where the compost is not exposed to the changing weather extremes (Guan et al., 2010; Vitosh-Sillman et al., 2017). The ability to compost confidently outdoors is going to be required for FAD control as most sites are not going to have the infrastructure to dispose of mortality on-site indoors. The most significant concern for outdoor composting is the compost piles reaching required temperatures in cold weather, such as US Midwest winters, the location of the majority of the countries pork supply (Oppedahl, 2020). Thus the objectives of this study were to analyze the preprocessing of carcasses for environmental risk of virus contamination and to ensure preprocessed compost reaches temperatures adequate for pathogen elimination in cold weather conditions.

Objectives:

1. Assess if the preprocessing (grinding) of carcasses for compost is a risk for aerosolization of swine viral pathogens
2. Determine if the composting of preprocessed swine carcasses under cold weather conditions will adequately kill common swine viral pathogens (PRRSV and PEDV)
3. Monitor internal temperatures to ensure preprocessed compost material produces enough heat under winter conditions to eliminate not only the surrogate viruses (PRRSV and PEDV) but also foreign animal diseases like ASFV
4. Analyze groundwater samples for potential leaching of swine viral pathogens from the composting material

Materials & Methods:

Animals:

Five-hundred sixty-six (566) feeder pigs were exposed to PRRSV, PEDV, and Seneca Valley Virus (SVV) by oral transmission route in the feed. The concentration of the challenged material was 1x10^5 Median Tissue Culture Infectious Dose (TCID50) for all pathogens. All animals were humanely euthanized by penetrating captive bolt, according to the American Veterinary Medical Association guidelines. Pigs were confirmed exposed by oral fluid PCR testing. Pigs were tested by serum PCR for PRRSV and by fecal swab PCR for PEDV and SVV to confirm infection. Forty (40) market weight animals from a local processing plant were also utilized in the study.

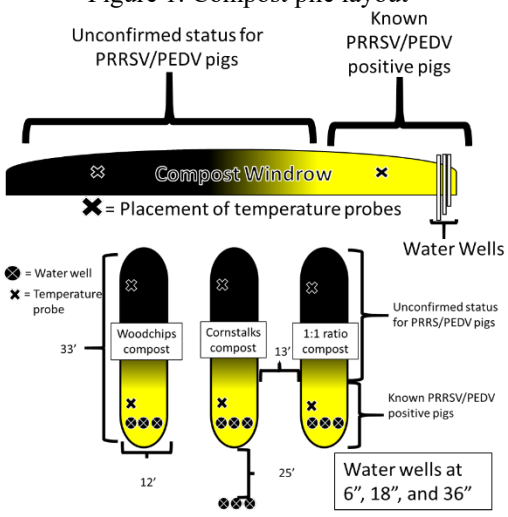
Diagnostics:

All diagnostic testing was completed at the Animal Disease Research and Diagnostic Laboratory at South Dakota State University according to standard diagnostic procedures. Compost grab samples were processed for testing as described in a previous study where 10g of compost is combined with 25ml of minimal essential media and processed in a stomacher blender for 2minutes at 230rpm (Vitosh-Sillman et al., 2017). Laboratory standard diagnostic procedures were used to test all compost, water, and air collection samples by oral fluid PCR.

Compost Windrow Formation:

All animals were preprocessed for compost by placement in a 750hp horizontal grinder (Rotochopper® FP-66 B-series, Rotochopper, INC, St. Martin, MN). Carcasses were placed with equal volumes of biomass during the preprocessing for compost. The carcass and carbon biomass blend were used in the formation of compost pile windrows. Three different biomass materials were used, and each type formed a different windrow: woodchips, cornstalks, and a 1:1 ratio of both. A one-foot base layer of each biomass type was laid down with the application of the processed carcass material over the top and then covered with the windrows represented biomass. Each windrow contained approximately 6200kg of pig carcasses, of which 544kg was the confirmed virus-positive surrogate pigs. The known processed positive pigs were placed together in the same region in each windrow biomass type for sampling, with the remaining pigs making up the rest in each pile. Dimensions and layout can be seen in Figure 1. Under the section of confirmed positive preprocessed carcasses of each biomass type, three water wells were placed to the depths of 6”, 18”, and 36” below ground surface and 25’ downhill from the compost piles. A 6” slotted polyvinyl chloride (PVC) piping was placed at the bottom of each well with Sodium bentonite placed around the top where it extended into the ground. Solid PVC was connected to the slotted section to remain accessible above compost material. PVC caps were placed over all exposed well openings to prevent external moisture contamination.

Figure 1: Compost pile layout



Water well sample collection:
Water samples were collected with a 3-way catheter valve with rubber tubbing long enough to reach the bottom of each well. A 60cc plastic syringe was used to create the suction to collect water from each well. The first water well collection attempt was made 5 days post windrow formation and continued weekly until the end of the study.

Aerosol Sampling:
Aerosol collection occurred with the use of six air collectors at a 200 liters/minute flow rate with a 60 minute run times per sample (Innovaprep, Drexel, MO). Collectors were positioned downwind from the horizontal grinder and ran for the entire preprocessing procedure. Two collectors were placed at each distance of 50yds, 100yds, and 150yds from the horizontal grinder. Samples were collected at the end of each one-hour runtime by filter 0.075% Tween 20/PBS wet-foam elution kits for diagnostic testing (Innovaprep, Drexel, MO).

Compost Temperature and Weather Monitoring:
The study was conducted in winter in the upper Midwest US for the provision of cold weather stresses. A local research weather monitoring station gathered daily high and low temperatures and daily rain and snowfall during the study (https://swroc.cfans.umn.edu/weather). Compost temperatures were measured by 3" long temperature probes placed at two locations in each pile (Figure 1). Compost temperature readings were taken daily for the first five days following windrow formation and then collected weekly until the completion of the study.

Compost Sample Collection:
Once preprocessing was complete, a sample respective of each biomass type was collected from the known disease positive material. Compost composite samples were collected daily for the first five days after windrow formation and then weekly until the end of the study. At each collection timepoint after windrow formation, two 10g grab samples of compost were collected by the careful deconstruction of the biomass cover layer to reach the processed carcass/biomass mix. One sample was from the outer "shallow" layer of the carcass/biomass mix, and one sample taken from approximately 3" deep into the processed material. Each 10g sample was placed into a sterile Whirl-Pak bag (Nasco, Fort Atkinson, WI, US) and stored at -80°C until testing. PCR testing was performed on all compost samples for the presence of PRRSV and PEDV.

Results:
Feeder pigs used in the study were confirmed exposed to PRRSV, PEDV, and SVV by oral fluid PCR testing. Samples were collected to verify infection before the preprocessing of carcasses began, but results were not available until after the pile formation was complete. PCR diagnostic results confirmed that the pigs were positive for PRRSV and PEDV by individual serum and fecal samples, respectively. However, PCR diagnostics were negative for SVV in the virus surrogate pigs used for windrow formation. PCR testing continued as planned for PRRSV and PEDV.

Objective 1) Assess if the preprocessing (grinding) of carcasses for compost is a risk for aerosolization of swine viral pathogens:

The entire preprocessing procedure took approximately 2.5hours to complete. Air collectors ran for three complete hours, half our past when the preprocessing was completed. PCR testing of the elution samples from the air sample collection were negative for PRRSV and PEDV.

Objective 2) Determine if composting the processed swine carcasses under cold weather conditions (winter in the Midwest US) will adequately eliminate swine viral pathogens (PRRSV and PEDV):

Table 1: PCR detection of PRRSV/PEDV in compost samples overtime

Day post windrow formation and virus tested		Woodchips[†]		Cornstalks		Combination	
Day 0	PEDV	29.60		29.03		27.85	
	PRRSV	29.07		27.76		26.90	
		Deep[‡]	**Shallow**	**Deep**	**Shallow**	**Deep**	**Shallow**
Day 1	PEDV	34.69	-[§]	33.19	NS[¶]	-	-
	PRRSV	35.13	-	28.51	NS	-	-
Day 2	PEDV	34.2	-	-	-	-	-
	PRRSV	34.15	-	-	-	-	-
Day 3	PEDV	-	35.59	-	-	-	-
	PRRSV	-	-	-	-	-	-
Day 4	PEDV	-	34.32	-	-	-	-
	PRRSV	-	27.12	-	-	-	-
Day 5	PEDV	-	-	-	-	-	-
	PRRSV	-	-	-	-	-	-
Week 2	PEDV	37.05	-	-	-	-	33.8
	PRRSV	-	-	-	-	-	-
Week 3	PEDV	-	-	-	-	-	-
	PRRSV	-	-	-	-	-	-
Week 4	PEDV	-	-	-	-	-	-
	PRRSV	-	-	-	-	-	-

[†]Three biomass types used for windrow formation include woodchips, cornstalks, and a combination of half woodchips and half cornstalks

[‡]Deep compost sample was at least 0.91m into the carcass processed material of the compost. Shallow samples collected from the outer section of the processed carcass material directly under the carbon source covering of windrow

[§] "NS" denotes a sample collected on which PCR diagnostics could not be performed

[¶] "-" represents a sample collected but for which PCR test results were negative

As seen in Table 1, all three compost biomass types were positive for the presence of PRRSV, and PEDV on the day windrows were formed (Day 0). After week two, all piles tested negative for viral nucleic acid going forward. Woodchips provided the most PCR positive results.

Objective 3) Measure temperature readings to ensure preprocessed compost material reaches critical temperatures under winter conditions to eliminate not only the surrogate viruses (PRRSV and PEDV) but also foreign animal diseases like ASFV:

Time post windrow formation	High (°F)	Low (°F)	Total Rainfall (in)	Total Snowfall (in)	Woodchips		Cornstalks		Combination	
					Probe 1 (°F)	Probe 2 (°F)	Probe 1 (°F)	Probe 2 (°F)	Probe 1 (°F)	Probe 2 (°F)

Day 0	23	13	0.02	0.2	46	46	74	66	52	52
Day 1	30	20	0	0	60	60	80	115	54	58
Day 2	44	26	0	0	**152**¶	**150**	86	**150**	132	110
Day 3	45	26	0	0	**168**	**166**	**146**	**154**	**156**	**160**
Day 4	38	26	0	0	**158**	**158**	**146**	**148**	**140**	**140**
Day 5	44	26	0	0	**165**	**162**	**144**	**144**	132	**152**
Week 2	50	17	0.18	0.8	45	45	132	134	100	**140**
Week 3	44	12	0.6	0.3	100	50	82	58	52	120
Week 4	51	12	0.3	0.2	52	50	78	54	48	48
Week 5	60	20	2.23	0.3	90	84	66	94	60	68

Table 2: Weather conditions† and temperature readings‡ of compost by biomass type§
†For days 0-5, the total rainfall and snowfall recorded as the total for that day. For weeks 2-5 in the table, the rain and snow recorded as the total for the week. High and low temperatures are reported daily for days 0-5, and the high and low temperatures are reported for the entire week for weeks 2-5
‡Two temperature probes were placed in each windrow type on opposite ends of the pile
§Three biomass types used for windrow formation include woodchips, cornstalks, and a combination of half woodchips and half cornstalks
¶Bolded values indicated temperature readings ≥140°C which is documented high enough to inactivate ASFV with 15-20minutes of exposure

For the inactivation of ASFV, a temperature of ≥140°F for 15-20 minutes is required (Mazur-Panasuik et al., 2019; United States Department of Agriculture, 2018). As seen in Table 2, even with cold weather conditions, all three windrows reach temperatures ≥140°F for multiple days.

Objective 4) Analyze groundwater samples for potential leaching of swine viral pathogens from the composting material:

Table 3: Real-time PCR ct results for PRRSV and PEDV testing on water samples collected from wells placed below each compost biomass types† and wells downhill at the designated depths

Week of collection after compost pile formation and virus tested		**Woodchips**			**Cornstalks**			**Combination**			**Downhill‡**		
		6”	**18”**	**36”**	**6”**	**18”**	**36”**	**6”**	**18”**	**36”**	**6”**	**18”**	**36”**
Week 1	PEDV	-§	-	-	-	-	-	neg	-	-	neg	-	-
	PRRSV	-	-	-	-	-	-	neg	-	-	neg	-	-
Week 2	PEDV	neg¶	-	-	-	-	-	neg	-	-	-	-	-
	PRRSV	neg	-	-	-	-	-	neg	-	-	-	-	-
Week 3	PEDV	neg	-	-	-	-	-	-	-	-	-	-	-
	PRRSV	neg	-	-	-	-	-	-	-	-	-	-	-
Week 4	PEDV	neg	-	-	-	-	-	-	-	-	-	neg	-
	PRRSV	neg	-	-	-	-	-	-	-	-	-	neg	-
Week 5	PEDV	-	-	-	**35.18**	-	-	neg	-	-	-	neg	-

	PRRSV	-	-	-	neg	-	-	neg	-	-	-	neg	-
Week 6	PEDV	-	-	-	-	-	-	-	-	-	-	neg	-
	PRRSV	-	-	-	-	-	-	-	-	-	-	neg	-

†Three biomass types used for windrow formation include woodchips, cornstalks, and a combination of half woodchips and half cornstalks

‡Wells placed 25' downhill from the three compost windrows

§ "-" signifies water collection was attempted but no sample present at that collection period

¶ "neg" signifies a negative PCR test result on the sample that collected for the well

As seen in Table 3, only one collected water sample tested positive by PCR for PEDV. PRRSV PCR testing was negative on all collected water samples. No virus material was found deeper than the 6" depth.

Discussion:

Although widely used as a standard method for mortality disposal, there are limited peer-reviewed reports on the survival of pathogens in the composing of swine carcasses (Wilkinson, 2007). A previous study on PEDV detection in compost only tested after the completion of the 1st and 2nd temperature cycles and did test routinely over time as performed in the current study (Vitosh-Sillman et al., 2017). The previous and current study demonstrated similar results with negative PEDV nucleic acid detection after the end of the first compost heat cycle (approximately 2 weeks after windrow formation). PRRSV virus detection overtime in compost has not been previously studied. The current study shows that PRRSV and PEDV have the potential to remain at detectable levels up to two weeks in compost. The presence of nucleic acid in on-site composting is an important consideration in disease control on farms, recognizing compost as a potential source of re-introduction of disease.

Concerning ASFV, survival in different matrices was found to be dependent on the moisture content (Mazur-Panasuik & Wozniakowski, 2020). A weakness of the current study is the moisture content of the compost biomass material was not evaluated before processing. The differences in moisture content is a potential explanation for the differences seen in this study with the extended detection of PEDV and PRRSV in woodchips compared to the compost windrows containing cornstalks. These differences in detection, however, were short-term as all three biomass types tested negative on consecutive weeks beginning three weeks after windrow formation. It is important to note that all biomass types evaluated in this study reached temperatures considered high enough to eliminate ASFV (15-20 minutes at ≥140°F) (Mazur-Panasuik et al., 2019; United States Department of Agriculture, 2018). The US Environmental Protection Agency classification for Class time-temperature pathogen reduction requires a composting temperature of 131°F for three consecutive days, which, as seen in Table 2, all windrows achieved (Costa & Akdeniz, 2019). Depending on the location of a mass depopulation and disposal, options in compost biomass type is imperative as woodchips may not be readily available in all areas of the country. Further evaluation of the differences compost biomass has on pathogen degradation overtime is needed.

In the event of carcass disposal for a FAD, environmental contamination is a concern in preventing further disease transmission. The monitoring of the air during the carcass preprocessing, revealed no PRRSV or PEDV detection by PCR in the air filter elution samples with a wind speed averaging 4-5mph. Under the conditions of the current study, aerosolization of pathogens during carcass processing appears to be a minimal risk for transmission. Further research is needed on different pathogen types, including DNA viral pathogens, to supplement these findings. Monitoring on the groundwater revealed a single PEDV PCR positive result and with no positive PRRSV results by PCR. The detection was only found at the shallow 6" depth. These results are similar to those observed with above-ground burial, carcasses buried directly under the topsoil, where the virus could be detected down 18" but not at 36" (B. Thaler, SDSU, personal communication, April 22, 2020). The minimal detection of pathogens in the current study suggests that groundwater contamination of low risk,

under the conditions of the study. In times or areas of greater rainfall, the potential of leaching viruses into the soil and groundwater may be higher (Chatterjee et al., 2013; Grisey et al., 2010). Given this study was performed in winter, the frozen ground may have prevented further pathogen leaching.

The current study confirms that the preprocessing of swine carcasses for composting is a viable potential option for disposal for depopulation events. Even in cold weather temperatures, compost reached heat levels high enough to denature common swine industry viruses (PRRSV and PEDV) and levels considered high enough to kill foreign animal diseases like ASFV. Under the conditions of the study, the risk of environmental contamination of air and groundwater were minimal. This minimal risk is vital not only for FAD eliminations but also for the disposal of regular daily mortality on farms during disease challenges. Although all three compost biomass types were proved adequate for virus elimination, there was an apparent short-term difference with pure woodchip biomass taking longer to test negative by PCR than the windrows containing cornstalks.

Further research is needed to assess if higher rainfall or greater wind speeds increases the risk of virus transmission or contamination. Validation of the ability of other compost biomass options to eliminate viruses would also be beneficial as woodchips and cornstalks may not be readily available in all areas. Evaluation of the carcass preprocessing before compost on other swine viruses, including SVV and a DNA virus pathogen, would further increase confidence in this technique for the elimination of infectious pathogens.

References:

Carriquiry, M., Elobeid, A., Swenson, D., & Hayes, D. (2020). Impacts of African Swine Fever in Iowa and the United States: Working paper 20-WP 600. Center for Agricultural and Rural Development, Iowa State University, Ames, IA USA.

Chatterjee, N., Flury, M., Hinman, C., & Craig, G.C. (2013). Chemical and physical characteristics of compost leachates. A review. Department of Crop and Soil Sciences: Washington State University.

Costa, T., & Akdeniz, N. (2019). A review of the animal disease outbreaks and biosecure animal mortality composting systems. Waste management, 90, 121–131. *doi:* 10.1016/j.wasman.2019.04.047

Erickson, L.E., Fayet, E., Kakumanu, B.K., & Davis, L.C. (2004). *Anaerobic digestion. In: Carcass Disposal: A Comprehensive Review*. Manhattan, KS: National Agricultural Biosecurity Center.

Ge, S., Li, J., Fan, X., Liu, F., Li, L., Wang, Q., Ren, W., Bao, J., Liu, C., Wang, H., Liu, Y., Zhang, Y., Xu, T., Wu, X., & Wang, Z. (2018). Molecular Characterization of African Swine Fever Virus, China, 2018. Emerging infectious diseases, 24(11), 2131–2133. *doi*: 10.3201/eid2411.181274

Grisey, E., Belle, E., Dat, J., Mudry, J., & Aleya, L. (2010). Survival of pathogenic and indicator organisms in groundwater and landfill leachate through coupling bacterial enumeration with tracer tests. Desalination, 261(1-2), 162-168. *doi*: 10.1016/j.desal.2010.05.007

Guan, J., Chan, M., Grenier, B.W., Spencer, J.L., Kranendonk, C., Copps, J., & Clavijo, A. (2010). Degradation of foot-and-mouth disease virus during composting of infected pig carcasses. The Canadian Journal of Veterinary Research, 74, 40-44. *PMID*:20357957

Kalbasi-Ashtari, A., Mukhtar, S., Hawkins, S., & Auvermann, B. (2005). Carcass composting for management of farm mortalities: A review. Compost Science & Utilization, 13, 180-193. *doi:* 10.1080/1065657X.2005.10702239

Mazur-Panasiuk, N. & Wozniakowski, G. (2020). Natural inactivation of African swine fever virus in tissues: Influence of temperature and environmental conditions on virus survival. Veterinary Microbiology, 242, 108609. *doi:* 10.1016/j.vetmic.2020.108609

Mazur-Panasuik, N., Zmudzki, J., & Wozniakowski, G. (2019). African swine fever - persistence in different environmental conditions and the possibility of its indirect transmission. Journal of Veterinary Research, 63, 303-310. *doi:* 10.2478/jvetres-2019-0058

Oppendahl, D.B. (2020). Improving Midwest agriculture and the environment. Chicago Fed Letter, Federal Reserve Bank of Chicago, issue 431. *doi:* 10.21033/cfl-2020-431

Rynk, R. (2003). Large animal mortality composting goes mainstream. BioCycle, 44 (6), 44-49.

Scudamore, J.M., Trevelyan, G.M., Tas, M.V., Varley, E.M, & Hickman G.A. (2002). Carcass disposal: lessons from Great Britain following the foot and mouth disease outbreaks of 2001. Revue Scientifique et technique (International Office of Epizootics), 21(3), 775-787. *doi*: 10.20506/rst.21.3.1377

United States Department of Agriculture. (2018). African Swine Standard Operating Procedure: Overview of Etiology and Ecology. Foreign Animal Disease Preparedness & Response. https://www.aphis.usda.gov/animal_health/emergency_management/downloads/sop/sop_asf_e-e.pdf

Vitosh-Sillman, S., Loy D. J., Brodersen, B. Kelling, C., Eskridge, K., & Schmidt, A. M. (2017). Effectiveness of composting as a biosecure disposal method of porcine epidemic diarrhea virus (PEDV)-infected pig carcasses. Porcine Health Management, 3, 22. *doi*: 10.1186/s40813-017-0068-z

Wilkinson, K.G. (2007). The biosecurity of on-farm mortality composting. Journal of Applied Microbiology, 102(3), 609-618. *doi*: 10.1111/j.1365-2672.2006.03274.x