RESEARCH REPORT
ANIMAL WELFARE

Title: **Validating water-based foam for the depopulation of swine – NPB #21-070**

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Industry Summary:
Increased global movement of people and animals increases likelihood of foreign animal diseases (FAD), like African Swine Fever (ASF), being introduced into the US swine population. Due to the risk of pathogen amplification in infected animals, swine on affected premises would need to be depopulated as soon as possible. In general, the goal for rapid depopulation is 24-hours or less after a confirmed positive (new detection) or presumptive positive (subsequent detections) diagnosis has been made. The size of most US commercial swine operations makes achieving this 24-hour goal difficult, with large sow units being especially problematic due to number of large animals (>200 kg). Most current methods for swine depopulation and disposal are not good candidates for mass depopulation in sow units because they are either time consuming (delivered at the individual level), create an unacceptable risk of disease dissemination for other swine populations (e.g. pathogen dissemination during movement of carcass material to disposal sites), or are practically infeasible to facility designs. Water-based foam is a preferred method for poultry depopulation, but limited testing of water-based foam has been conducted on swine under field conditions and guidelines for their use under emergency situations are lacking. We sought to further investigate the effects of water-based foam depopulation to prepare the swine industry for mass depopulation and disposal events. The overall objective of this proposed mixed-methods project is to fill the critical gaps in scientific evidence to support an application for AVMA approval of water-based foam for depopulation of all ages of swine. Our findings demonstrate that depopulation with water-based foam reliably induced rapid unconsciousness (average of 2 minutes) and subsequent brain death in all age pigs. After 5 minutes in the foam, none of the pigs were recoverable, which means the process can be used to rapidly depopulate groups of swine from a modern swine facility. Additionally, 86.7% of industry stakeholders who observed depopulation of pigs with water-based foam indicated that water-based foam was a better depopulation method than any of currently approved methods (e.g. captive bolt, electrocution, CO2). Our findings support efforts to seek conditional AVMA approval of water-based foam as an emergency depopulation option for all ages of swine.

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Key Findings:

- On average, pigs treated with water-based foam transitioned to an unconscious state at 2 minutes post-application; the average time to onset of brain death was 3 minutes and 10 seconds.
- The minimum time dwell time under the foam in order to reach reliability and irreversibility was 5 minutes. While 5 minutes was 100% successful for both nursery pigs and sows, we are recommending 7.5 minutes out of an abundance of caution.
- After viewing application of water-based foam to a group of pigs, 86.7% of observers indicated water-based foam was a better depopulation method than any of currently approved methods (e.g. captive bolt, electrocution, CO_2)
- Observers experienced few to no negative long-term mental health impacts after observing depopulation of swine with water-based foam.

Keywords: swine depopulation, water-based foam, cull sow depopulation, nursery pig depopulation, EEG

Scientific Abstract:
Swine populations are susceptible to infectious diseases, and those instances urgent responses including large-scale depopulation may be required to control and prevent farm-to-farm transmission during outbreaks. The American Veterinary Medical Association (AVMA) recommends a number of methods for swine depopulation, but their efficiency for large populations is debatable. In this study, water-based foam (WBF), an AVMA-approved method in poultry, was adapted to swine. The objectives of the study were to describe "time to animal unconsciousness" and "time to non-recoverability" using WBF; and to provide a recommendation on the duration of WBF foaming under field conditions. Additionally, the perceptions of people observing application of WBF to pigs was also assessed.

Two trials (Trial 1 and 2) were conducted for a stepwise evaluation on the use of WBF in swine. Trial 1 assessed time to unconsciousness by immersing 72 feeder pigs (6 pigs for 12 replicates) in WBF for 6 time periods (2.5, 5, 7.5, 10, 12.5, and 15 minutes post-fill (MPF); 2 replicates per time period). The recoverability of foamed pigs was assessed via different levels of vital signs, and the time period for which no "Level 2" were present was selected for Trial 2. In Trial 2, 135 cull sows (3 replicates of 45 sows) were loaded into an adapted rendering dump trailer (40 × 8.5 × 6 in) and immersed in WBF for the period suggested in Trial 1. Activity bio-loggers (Star-Oddi©) were implanted in 6 sows in each replicate to measure animal movements. The last movement was determined by the last external acceleration measurement beyond the third quartiles + 1.5 times the interquartile range. For both trials, pigs with any signs of consciousness were immediately euthanized by captive bolt.

In Trial 1, kicking, respiratory rhythm, and convulsions were absent while regular gasping and corneal reflex were observed respectively in 3 and 12 pigs at 2.5 minutes MPF. Consciousness signs > "Level 1" were absent at ≥ 5 MPF; thus, sows were immersed for 5 MPF in Trial 2. In Trial 2, the mean durations between end of trailer WBF fill and last animal movement (min:sec) as per bio-loggers were 1:332:40 (SD=1:31), 1:102:26 (SD=1:11), and 0:221:58 (SD=0:47) for replicates 1, 2, and 3 respectively. All sows were deemed unconscious after unloaded from the trailer.

In a third trial, EEGs were performed on 12 feeder pigs as they were depopulated with WBF. The average time to onset of transitional period (i.e. unconciseness) was approximately 2 minutes; and the average time to onset of isoelectric period was 3 minutes and 10 seconds.

Invited stakeholders (n=33) completed pre- and post- surveys on perceptions regarding animal behavior, methodology, and likelihood of field success. The WBF depopulation process was applied in cull sows. Comparing pre- to post- survey responses, the actual time (in minutes) to fill the trailer with foam (5.0±12.0 vs. 1.0±0.7), stop hearing animal vocalizations (5.0±6.5 vs. 0±1.0), and stop hearing animal movements (7.0±6.0 vs. 2.0±0.25) were all shorter than anticipated ($P<0.001$). Additionally, the majority of participants indicated WBF was a better method than currently approved depopulation methods (e.g. captive bolt, electrocution, CO_2), which was significantly higher than before they observed the process (86.7% vs. 56.3%; $P=0.008$) Additionally, observers reported few to no negative long-term mental health impacts after observing depopulation of swine with water-based foam.

Based on our studies, WBF could be an attractive method for emergency depopulation in swine. Given our assessment of time to unconsciousness in feeder pigs and our field validation in sows, we recommend that 7.5 minutes of submersion should be used to ensure unconsciousness and subsequent death. Our findings support efforts to seek conditional AVMA approval of water-based foam as an emergency depopulation option for all ages of swine.

Introduction:

Modern swine production coupled with expanding global movement of people and animals increases the chance of introduction and spread of pathogens in US swine populations. Disease outbreaks cause negative impacts on animal health and welfare, public health and perception, the

environment, food supply, and the economy. To minimize such effects, control plans are needed. As an example, African Swine Fever (ASF) is a severe viral disease affecting domestic and feral pigs, for which currently there is no commercially available vaccine. Although the US swine herd is free of ASF, there is current and ongoing transmission throughout China and parts of Europe (OIE, 2020). Because the ASF virus can be transmitted by numerous modes of transmission, preparation of contingency plans remains a high priority for the US swine industry. These plans must include detailed steps for depopulation of large numbers of swine in a short timeframe.

Even though there are informative guidelines developed by AVMA on swine depopulation and euthanasia (AVMA 2019, AVMA 2020), applying recommended measures to large populations of swine under field conditions while assuring rapid, humane destruction of animals and the provision of safe and mental health efficacious protocols for humans remains a recognized challenge. As recommended by the AVMA, developing and testing a plan before an incident occurs becomes imperative. The goal of best practice depopulation systems is to minimize or eliminate animal anxiety, pain, and distress before the loss of consciousness. Thus, when evaluating depopulation systems, both the induction of unconsciousness and handling/restraint processes must be considered (AVMA 2019). Current preferred depopulation methods include physical methods (non-penetrating captive bolt, penetrating captive bolt, electrocution, manual blunt force trauma, and movement to slaughter) and inhaled methods (carbon dioxide and anesthetic overdose). However, most of these methods are not good candidates for mass depopulation because they are either time consuming (delivered at the individual level), and/or would represent a high risk of disease dissemination to other swine (e.g. movement to slaughter). Inhalation methods offer significant promise because they allow multiple animals to be depopulated simultaneously. Nonetheless, limited testing of inhalation and alternative methods has been conducted under field conditions and outcomes have not yet provided sufficient data to develop firm guidelines for use in emergency situations (Meyer and Morrow, 2005; Kinsey et al., 2016; Rice et al., 2014).

As emerging disease control plans are discussed and prepared, economic and animal welfare considerations are usually at the forefront. However, the potential for social impacts should not be overlooked (Evans, 2006). Social impacts refer to indirect health consequences at the individual, family or community level; that could involve a wide range of personnel such as veterinarians, farm owners and animal caretakers, and first responders. Detrimental psychological and emotional impacts of animal euthanasia and depopulation have been reported in field personnel involved with the implementation and acts associated with the eradication of Foot and Mouth Disease in The Netherlands, the United Kingdom, and Japan, as well as eradication of bovine spongiform encephalopathy in Canada (Olff et al., 2005; Evans, 2006, Hibi et al., 2015). Of note, most of these publications reflect either expert opinion on impressions on a method's impact on personnel wellbeing (McReynolds and Sanderson, 2014), or assessments of post-traumatic stress symptoms conducted several years after the disasters (Olff et al., 2005; Evans, 2006, Hibi et al., 2015).

However, by using water based foam depopulation, both a physical and mental barrier is created between personnel and individual animals. This distance may be beneficial to swine welfare by reducing malpractice due to physical and mental exhaustion and by decreasing undesirable side effects such as compassion fatigue and post-traumatic stress symptoms in personnel.

Furthermore, details on the depopulation methods and specific aspects that contributed to the traumatic impressions are not assessed, preventing a more complete understanding of which specific parts of the intense experience are responsible for causing the trauma. This information, if collected proactively, could help facilitate timely interventions to prevent more serious disorders after the incident.

When a foreign animal disease is introduced to the US swine herd, economic impact to producers is reduced by preventing further disease transmission. Rapid depopulation of infected and exposed animals may help maintain continuity of business for non-infected animals and non-contaminated animal products. As we know, one solution does not fit all situations in the U.S. pork industry. Water based foam may provide an additional solution for an emergency depopulation event. Results summarized following completion of each phase in the proposed study will provide the US

pork industry with new science, identify opportunities and challenges, and ultimately support or refute the hypothesis that large-scale depopulation can be performed in an efficient, cost effective, and disease containment strategy applicable across the swine industry.

Objectives:

The overall objective of this proposed mixed-methods project is to fill the critical gaps in scientific evidence to support an application for AVMA approval of water-based foam for depopulation of all ages of swine.

Objective 1: Quantify the time required for water-based foam to induce loss of consciousness and describe behavior of the animal during that time

Objective 2: Assess the reliability and irreversibility of water-based foam to result in death of nursery pigs and sows

Objective 3: Describe personnel's perception of swine depopulation using water-based foam, and identify areas of concern during method execution to diminish the potential for detrimental psychological and emotional impacts.

Materials & Methods:

This study was approved through The Ohio State's Institutional Animal Care and Use Committee protocol number 2020A00000036.

Foam Generation:
Foam was generated using a gas-powered water pump and three medium-expansion foam nozzles. For each nozzle, a 2-inch suction hose will be connected to the pump inlet and placed in water reservoir containing 1% foam-water solution (PHOS-CHEK WD881 Class A foam concentrate). The pump outlet was connected with a 1.5-inch diameter firefighting hose attached to a medium-expansion foam nozzle (AWG, HMFN-M4). This setup provided a 50:1 expansion ratio. Foam was applied until it reaches the top of the vessel.

Objective 1: Brain activity
Twelve nursery pigs were used for this objective. Prior to foam application, each pig was fitted with six electroencephalogram (EEG) electrodes, and a waterproof backpack containing a portable EEG transmitter. Individually, each pig was placed in a sling within a plastic bulk container and foam was applied. EEG traces were collected starting 5 minutes prior to the application of foam and continue for 15 minutes post application of foam. Following the 15 minutes, the pigs were removed from the foam and death was confirmed. EEG traces were sent to Dr. Jorge Vidaurre, Nationwide Children's Hospital, and analyzed for time to loss of consciousness using previous criteria defined by Gibson et al. (2019). In brief, EEG traces were categorized into five categories: 1) Movement artefact; 2) Normal EEG; 3) Transitional EEG; 4) HALF EEG (High Amplitude, Low Frequency); and 5) Isoelectric EEG. Category 2 was similar to baseline; category 3 was defined as an amplitude of less than half of that of the baseline; and category 5 was defined as a trace with amplitude 12.25% of that of baseline EEG.

Objective 2: Reliability and irreversibility
Prior to foam application, 10 pigs had a subcutaneous data logger (Star-Oddi,) placed caudal to the left or right triceps. Water-based foaming agents will be applied in a 47"L x 47"W x 40"H', closed top, opaque plastic bulk container used successfully in previous approved water-based foam depopulation studies. Six nursery pigs (40 to 60 lbs), providing ~2.66 sq. ft. of available surface space per pig, were placed in the container and foamed within a replicate. Within a replicate, a unique colored nylon rope was affixed (tied and taped) to one rear leg of each pig to allow retrieval of one pig at each of 6 time points examined (2:30, 5:00, 7:30, 10:00, 12:30, and 15:00 (min:sec)). Upon each removal, pigs were laid on a hard surface and observed for stun efficacy using an adaption for the protocol described by

Atkinson et al. (2012), available in Figure 1. All pigs were evaluated for presence of a heartbeat and confirmed dead prior to disposal. The presence of a level 2, 3 or 4 stun response within a replicate resulted in removal of the time point from further replication. Power analyses indicated 12 replications (6 pigs per replicate, n=72 nursery pigs total) were required.

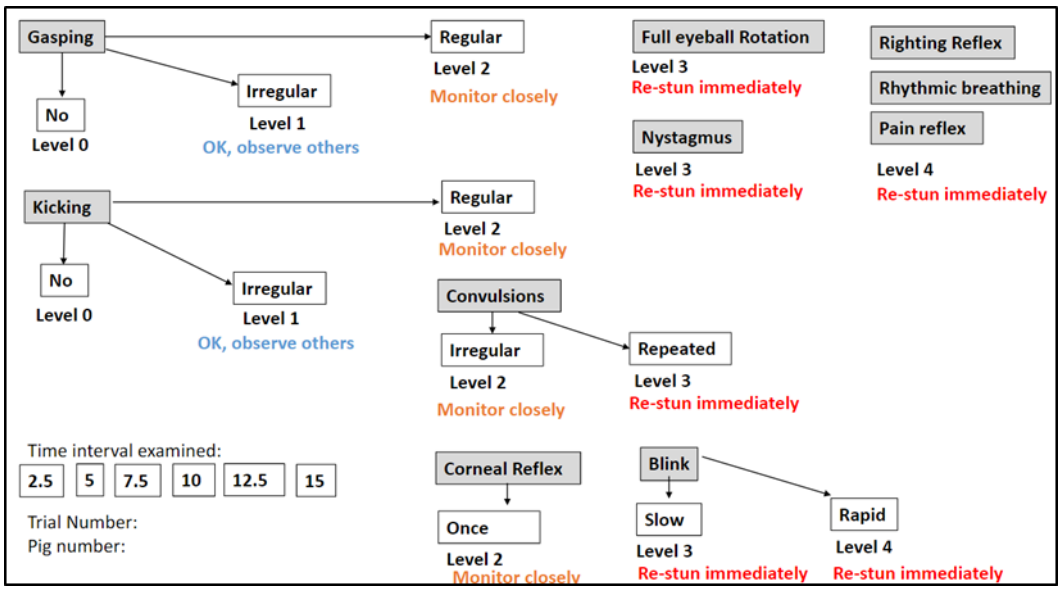

Figure 1. Flowchart used to assess non-recoverability designed by the research team

Using time/treatment success rate of 100% identified in the nursery pig study, the identified dwell time was further evaluated in 75 cull sows. Prior to the trial, activity bio-loggers (Star-Oddi©) were implanted in 6 sows in each replicate to measure animal movements. In three replicates, sows were placed in a sealed trailer and foam applied to the group. At the end of the designated time point, the trailer was lifted to allow carcasses to spread, and a team of trained investigators evaluated individual sows for stun efficacy. Bio-loggers were then recovered, and data was analyzed. The last movement was determined by the last external acceleration measurement beyond the third quartiles + 1.5 times the interquartile range.

Objective 3: Stakeholder perceptions
Swine stakeholders were recruited through the investigator's professional network to watch replicates from the swine foam depopulation. These individuals included veterinarians, animal health officials, researchers, extensionist, and students. Those agreeing to participate in this study completed two short questionnaires (10-15 minutes each): one prior to and one after the depopulation trial. Initial drafts of the questionnaires were constructed using previous surveys designed to assess the degree of distress caused by depopulation and were adapted after consultation with a professional with mental health specifically in the agricultural field (Dr. Josie Rudolphi, University of Illinois). Areas covered on the questionnaire included the respondent's role and previous experience with swine euthanasia, impressions on the effectiveness of water-based foam as it relates to animal welfare (e.g. time to cessation of movement, escape attempts, etc.) and timely accomplishment of depopulation (e.g. time for container fill with foam, time to death); and opinion on the likelihood of success if this method were to be applied in large scale. Information was captured anonymously and analyzed in a manner to protect participant's confidentiality. A follow-up survey was also requested at least one month after the trial in order to capture long-term impressions.

Results:

Objective 1: Brain activity
Summarized EEG results can be found in Figure 2. The average time to onset of transitional period was approximately 2 minutes; and the average time to onset of isoelectric period was 3 minutes and 10 seconds. The pig with the longest time to onset of transitional period was approximately 3 minutes, and the pig with the longest time to onset of isoelectric period was approximately 5 minutes.

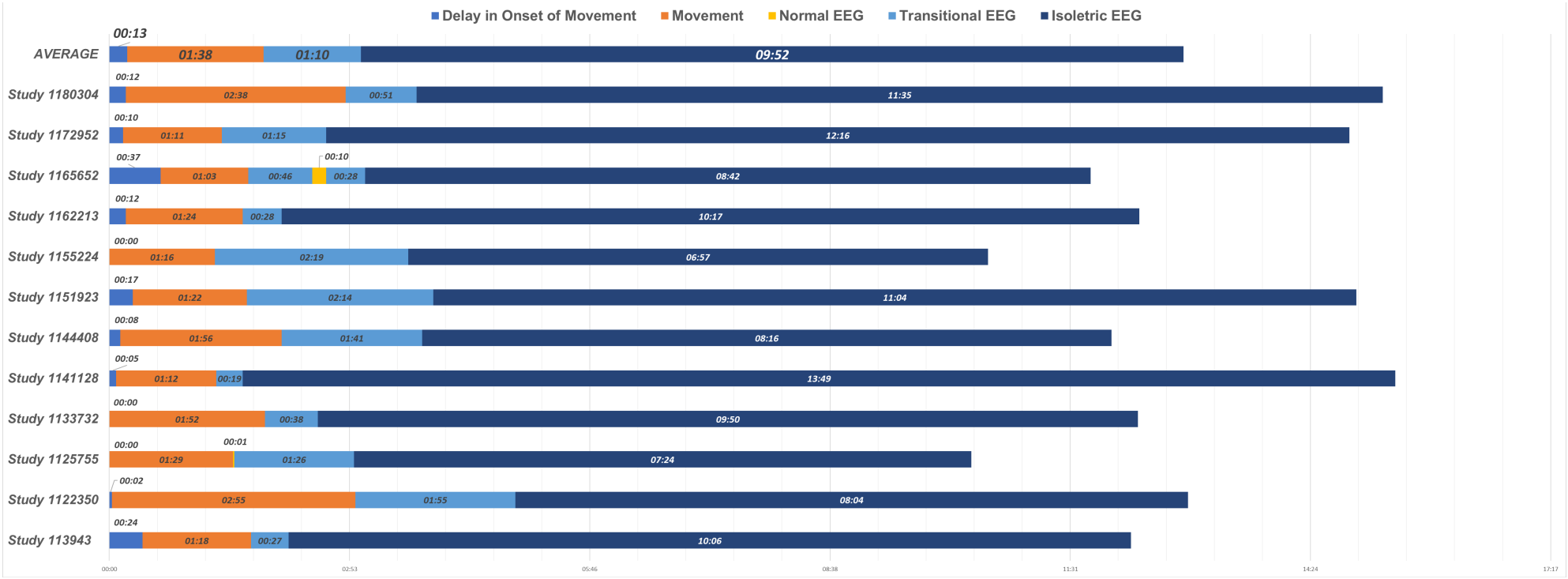

Figure 2. Characteristics of the EEG in foamed individual subjects (foam started in time point 0). Bars represent time to onset of movement artifact, transitional EEG, normal EEG and isoelectric EEG, according to the legend. Bars stopped when last heartbeat was recorded. Average values ae represented in the top bar.

Objective 2: Reliability and irreversibility
Results from the assessment is available in Table 1. Based on these results, the minimum time defined to keep animals under the foam in order to reach reliability and irreversibility of this method was 5 minutes.

	Time point (in minutes)					
Measurement	2.5	5	7.5	10	12.5	15
No Corneal Reflex	0 (3 score 2, 9 score 3)	100 (12)	100 (12)	100 (12)	100 (12)	100 (12)
No Respiratory rhythm	100 (12)	100 (12)	100 (12)	100 (12)	100 (12)	100 (12)
No Heartbeat	0 (all had heartbeat present)	17 (10 had faint heartbeat that ceased)	83.3 (2 pigs had faint heartbeats that ceased)	100 (12)	100 (12)	100 (12)
No Convulsions	100 (12)	100 (12)	100 (12)	100 (12)	100 (12)	100 (12)
No Gasping	0 (12) (3 scored 2; 9 score 1)	100 (4 had score 1 that ceased)	100 (12)	100 (12)	100 (12)	100 (12)
No Kicking	100 (12)	100 (12)	100 (12)	100 (12)	100 (12)	100 (12)
Captive Bolt Needed	100 (12)	0 (12)	0 (12)	0 (12)	0 (12)	0 (12)

Table 1. Time to non-recoverability in nursery pigs. Proportion of pigs or replicated; in % (n) deemed unconscious for individual measurements (not presenting considerable signs for the assessed items).

For the cull sow trials, 75 cull sows were immersed in WBF for the period suggested in Trial 1 (5 minutes). The mean durations between end of trailer foam fill and last animal movement (min:sec) as per bio-loggers were 2:40 (SD=1:31), 2:26 (SD=1:11), and 1:58 (SD=0:47) for replicates 1, 2, and 3 respectively (Figure 3,4,5). All sows were deemed unconscious after unloaded from the trailer, corroborating findings in nursery pigs.
In conclusion for this objective, we recommend 7.5 minutes as the appropriate dwell time in water-based foam for all ages of swine. While 5 minutes was 100% successful for both nursery pigs and sows, we are recommending 7.5 minutes out of an abundance of caution.

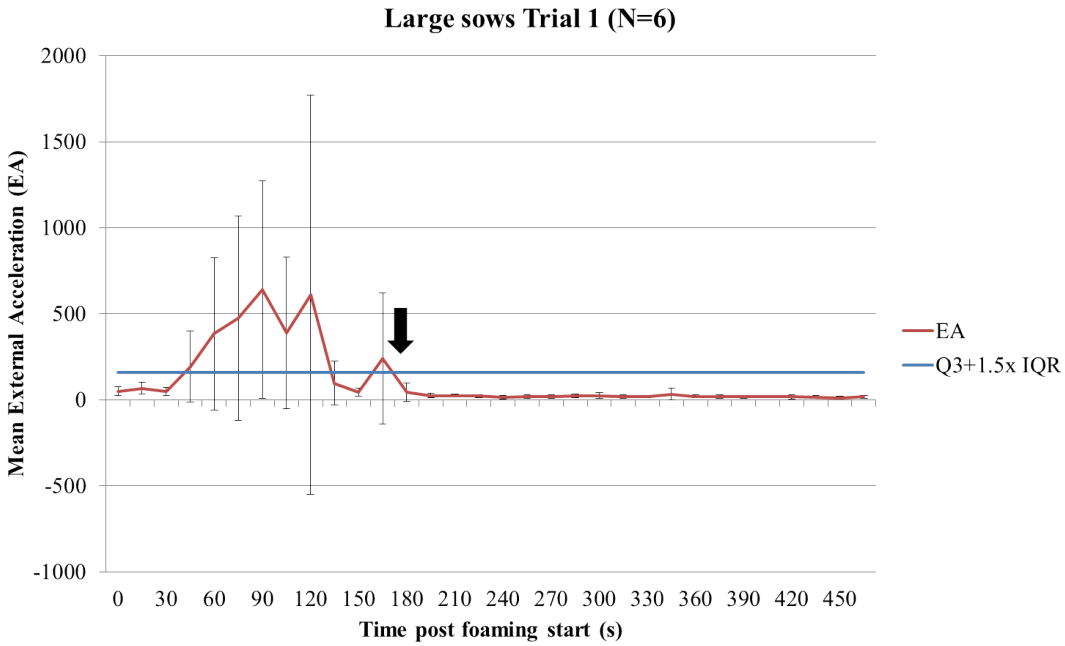

Figure 3. Average time (s) to cessation of animal movement in cull sows using water based foam. Arrow indicates time window after the last animal movement was detected using activity data (external acceleration) from Star-Oddi® bio-loggers.

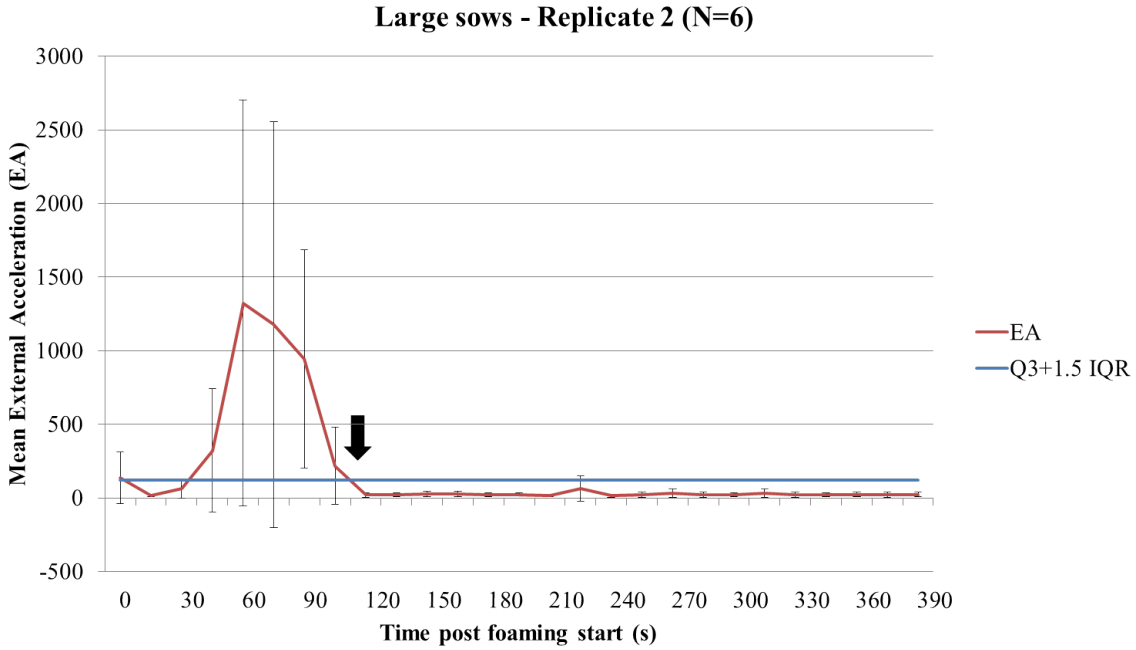

Figure 4. Average time (s) to cessation of animal movement in cull sows using water based foam. Arrow indicates time window after the last animal movement was detected using activity data (external acceleration) from Star-Oddi® bio-loggers.

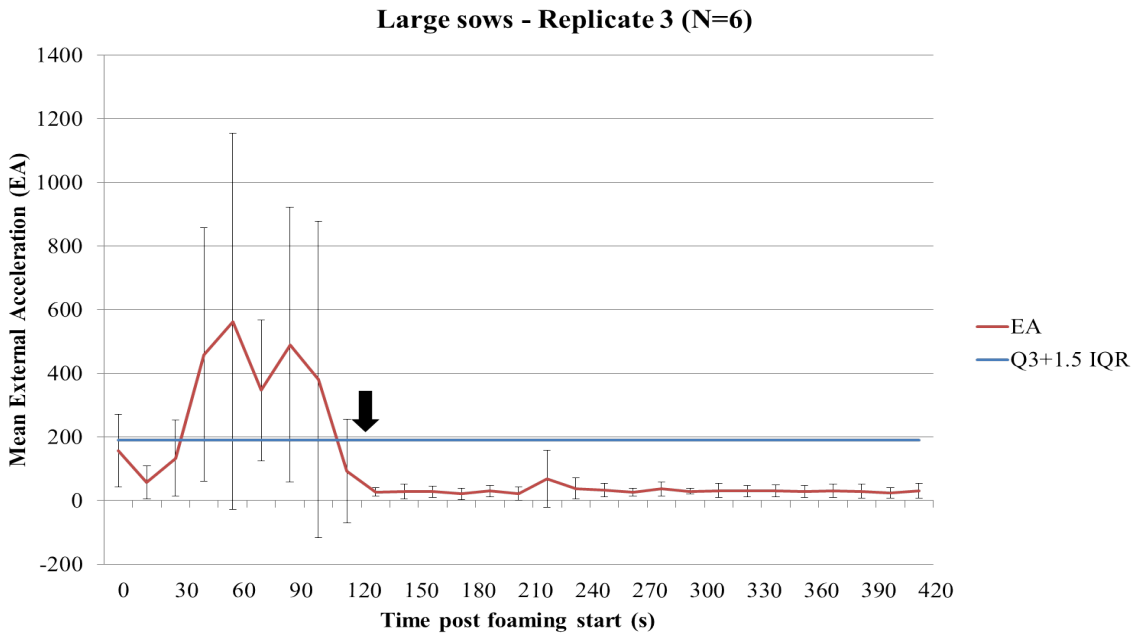

Figure 5. Average time (s) to cessation of animal movement in cull sows using water based foam. Arrow indicates time window after the last animal movement was detected using activity data (external acceleration) from Star-Oddi® bio-loggers.

Objective 3: Stakeholder perceptions

A total of 33 participants completed the pre-trial survey, and 30 completed both the pre- and post-trial survey. From those participants, the main self-identified role was as animal health officials (n=11), educators/researchers (n=9), veterinarians (n=8) and other (n=5), indicating 0 to 43 years of experience in the swine industry (10.0±23.0 [median ± IQR]).

Comparing pre- to post- survey responses, the actual time (in minutes) to fill the trailer with foam (5.0±12.0 vs. 1.0±0.7), stop hearing animal vocalizations (5.0±6.5 vs. 0±1.0), and stop hearing animal movements (7.0±6.0 vs. 2.0±0.25) were all shorter than anticipated ($P<0.001$). Additionally, the majority of participants indicated WBF was a better method than currently approved depopulation methods (e.g. captive bolt, electrocution, CO_2), which was significantly higher than before they observed the process (86.7% vs. 56.3%; $P=0.008$).

Results from our long-term survey, completed by 14 participants, is presented in Table 2. These surveys were completed 1-4 months after the trial had been observed. Our results showed that none of the participants reported feeling “jumpy or easily startled”, or “irritable with angry outbursts” after the depopulation trials; 85% of the respondents reported not having experienced any repeated disturbing memories, thoughts or images from the depopulation trials (15% or 2 respondents stated “a little bit”); and 92% of participants reported not feeling upset when reminded of the population trial, and no difficulty in concentrating in regular tasks (1 participant reported “a little bit” for both of those questions). Overall, participants appear to experience few to no negative impacts long-term after observing depopulation of swine using WBF.

	Not at all	A little bit	Moderately	Quite a bit	Extremely
Disturbing memories/ thoughts?	12	2	0	0	0
Feeling upset from memories?	13	1	0	0	0
Avoiding activities?	14	0	0	0	0
Irritable or angry?	14	0	0	0	0
Difficulty concentrating?	13	1	0	0	0
Jumpy or easily startled?	14	0	0	0	0

Table 2. Summary of responses (number) from participants 1-4 months after watching the depopulation trial using water-based foam.

Discussion:

Water-based foam is a preferred method for poultry depopulation because it causes a rapid death and is relatively easy apply. Our results show that water-based foam resulted in a rapid death of piglets and sows that was quickly non-reversible. Additionally, the perceptions and attitudes of stakeholders who observed the application of water-based foam was very positive. While water-based foam is approved for depopulation of poultry, it is not currently approved for use in swine, which creates a hurdle for the pork industry. Overall and combined, our findings support efforts to seek conditional approval of water-based foam as an emergency depopulation option for swine.

Given its use with poultry, USDA already has water-based foam generating units and foam concentrate in the National Veterinary Stockpile, which makes foam an attractive agent for swine. Historically, foam has not been feasible in swine due to slatted floor housing; however, use of a trailer provides new opportunities. Our 2020 study showed that water-based foam in modified rendering trailers is a viable depopulation option for mature swine. Additionally, subsequent pilot studies showed equal success with weaner and feeder pigs.

Water-based foam is relatively inexpensive. The only specialized equipment needed is a high-pressure water pump and expansion foam nozzle. Trash pumps or firefighting pumps are available in nearly every community across the US, and foam expansion nozzles are under $1,000 each, which means there is little investment cost. Foam concentrate is approximately $25\gallon, and 3 gallons of foam concentrate mixed with 300 gallons of water will expand fill an entire semi-trailer. Additionally and unlike CO_2, the agent can be stockpiled for the emergency ahead of time and water should be readily available on every swine farm. Water-based foam is a group application depopulation method, which makes it well suited for rapid, large-scale depopulation of nursery pigs through adult swine.

Certainly, using a water-based method can present challenges during cold weather, but the challenges can be overcome by using techniques employed by firefighters globally (continual

water circulation, keeping fuel powered engines running, spraying caps and plugs with antifreeze, etc.).

Our results support a recommendation of 7.5-minute dwell time, which means the rate-limiting step in water-based foam depopulation is the speed with which animals can be loaded into the trailers.

Presentations:
(peer reviewed) Williams, T. J., Cheng, T.-Y., Campler, M.R, Bowman, A. S., Moeller, S., Kieffer, J., Arruda, A. G. Swine industry stakeholder perception on depopulation using water-based foam. 2021 Conference of Research Workers in Animal Diseases (CRWAD), Chicago, IL. Dec 05-08, 2021. (oral, presented by DVM-MPH student).