SWINE HEALTH INFORMATION CENTER
FINAL RESEARCH GRANT REPORT

Assessing distribution and mitigation of Senecavirus A, a foot and mouth disease surrogate, in a swine feed mill (Project #19-148 SHIC)

Principal Investigator: Cassandra Jones, PhD
Department of Animal Sciences & Industry
Kansas State University
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Contact:
Cassandra Jones, PhD
Associate Professor
Department of Animal Sciences & Industry
Kansas State University
Manhattan, KS 66506
Phone: 785-532-5289
Emal: jonesc@ksu.edu

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Industry Summary: Kansas State University
Environmental monitoring is commonly used in pharmaceutical, human food, and pet food manufacturing facilities manufacturing as an indicator of pathogenic bacteria in the product. A correlation between the presence of *Salmonella* spp. and Enterobacteriaceae within feed mills has been demonstrated, but little information is available on how the presence of Enterobacteriaceae (EBAC) correlates with viral pathogen presence, especially on farms or in feed mills. The purpose of this study was to identify Enterobacteriaceae presence in the feed manufacturing facilities of a multi-farm system experiencing a viral outbreak as a method of identifying biosecurity gaps.

Three separate feed manufacturing facilities (Sites 1-3) were evaluated and sampled for this study, with a biosecurity evaluation and audit performed during each visit. A total of 573 samples were taken over the course of four days, with 381 of those samples consisting of feed ingredient or finished feed, and the remaining 192 samples environmental swabs, collected across the 4 sites. Each swab was assigned one of four zones, including direct feed or ingredient contact surfaces (Zone 1), close proximity non-contact surfaces (Zone 2), non-contact surfaces without close proximity (Zone 3), and transient surfaces, such as moveable tools, employees, and vehicles (Zone 4). Swabs taken from a fourth facility, a multiplier farm, were assigned zones based on proximity to pigs. This included direct feed-contact surfaces (Zone 5), direct pig-contact surfaces (Zone 6) including pen flooring, pen walls, feeders, and waterers (pig contact), and non-pig contact surfaces (Zone 7) including employee walkways, work areas, feed storage, and fans (non-pig contact).
After collection, samples were shipped to the Iowa State Veterinary Diagnostic Laboratory, and the three types of bacteria with largest growth for each sample were identified and reported by assigning a growth index value. Bacterial growth results were

assigned an index value of either 0, 1, 2, 3, or 4 based on reported growth, representing no, few, low, moderate, or high growth, respectively.
Maps of EBAC levels per facility are shown in Figures 2, 3, and 4. Audit scores for each facility were 83%, 67%, and 42% for Site 1, 2, and 3, respectively. Site 1 utilized locked exterior doors, required employees to change clothes and shoes prior to entry, and had handwashing stations located inside the doorway. The scale was located within a fenced perimeter, and was used to weigh company-owned pigs occasionally. In Site 1, the scale, receiving pit, finished feed bin, and finished feed truck were the only feed-contact surfaces with detected EBAC. At Site 2, exterior doors were not locked and handwashing stations not used except for restroom purposes, but employees changed clothes and shoes prior to entry. There was no perimeter fence and the scale was routinely used to weigh animals. There was a moderate quantity of EBAC detected in all feed contact surfaces tested, with high levels on the floor of the manufacturing area. At Site 3, exterior doors were not locked and handwashing stations not used except for restroom purposes, but employees changed clothes and shoes prior to entry. There was no perimeter fence and the scale was routinely used to weigh company-owned animals, as well as those from other within the region. While it was difficult to obtain samples from feed contact surfaces in Site 3, those collected all had high levels of EBAC.

There was significant evidence of a weak correlation ($r = 0.201$, $P \leq 0.0001$) between EBAC presence and site. There was evidence of moderate correlation noted ($r = 0.463$, $P \leq 0.0001$) between the zone and presence of EBAC, but no evidence of correlation ($r = 0.028$, $P > 0.05$) between zone a presence of fecal indicator bacteria.
Clearly, compliance with biosecurity protocols had a substantial impact of Enterobacteriaceae prevalence and distribution throughout the feed mill. As facilities begin to transition biosecurity from the farm to the feed mill, using environmental monitoring to evaluate risk for biosecurity gaps, as well as success in their mitigation, will be useful and necessary.

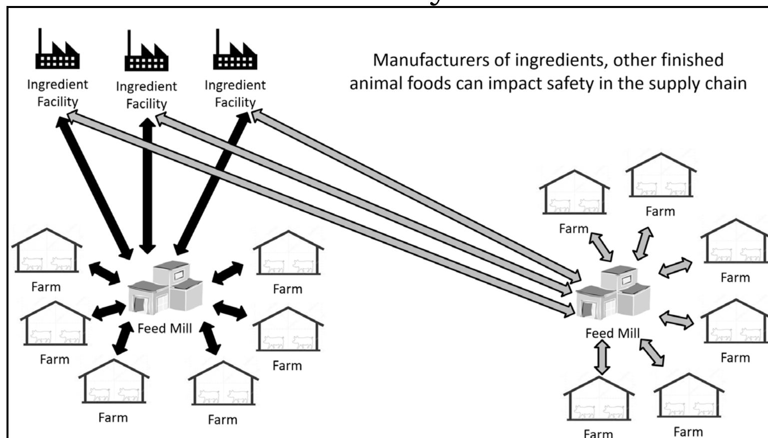

Figure 1.

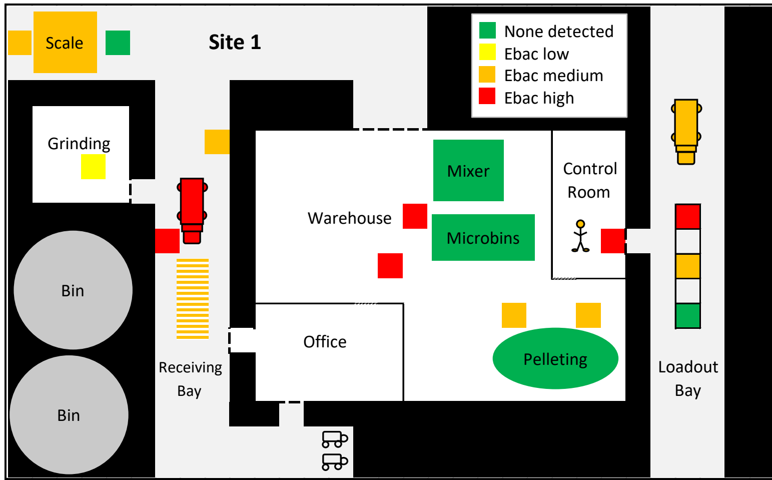

Figure 2.

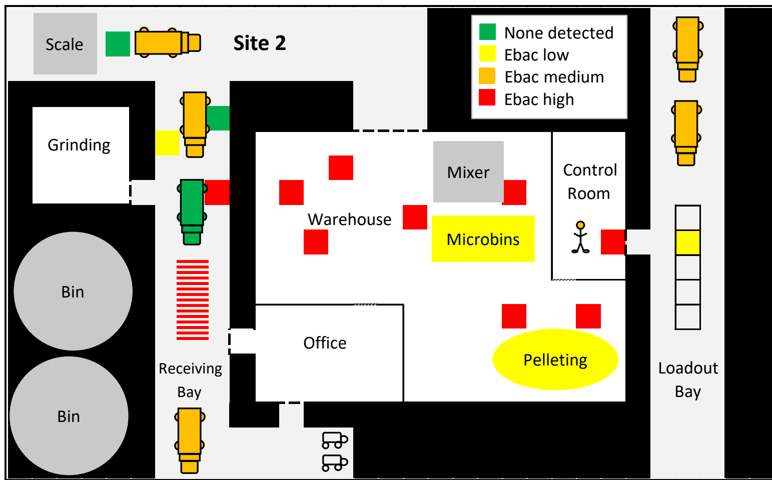

Figure 3.

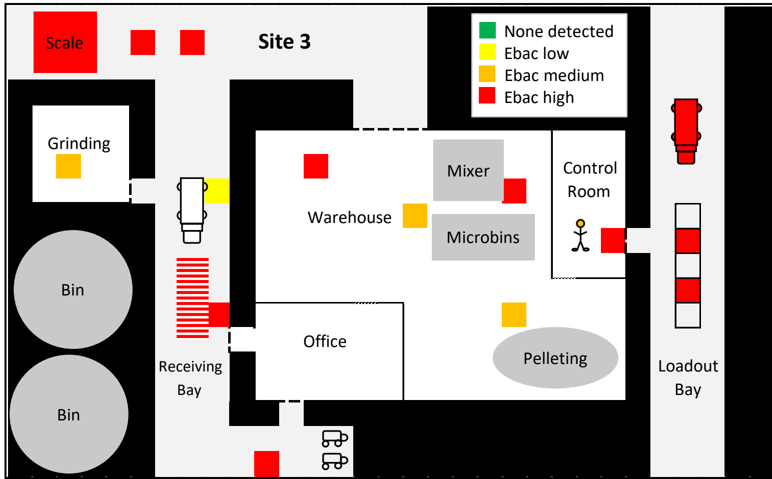

Figure 4.

Scientific Abstract: Three separate feed manufacturing facilities (Sites 1-3) were evaluated and sampled for this study, with a biosecurity evaluation and audit (posted at ksuswine.org) performed during each visit. Each mill offered its own biosecurity challenges, either with the normal operating procedures or required tasks to be performed within facility limitations. A total of 573 samples were taken over the course of four days, with 381 of those samples consisting of feed ingredient or finished feed, and the remaining 192 samples environmental swabs, collected across the 4 sites.
Feed ingredient and finished feed samples were collected using single-use plastic tubs. For each separate item, 10 individual samples were collected initially. For bulk-storage products, samples were either drop-collected or grabbed at multiple times while being conveyed. For bagged products, samples were obtained from each of 10 different bags onsite. Each sample was kept separate for individual analysis, with an additional blended composite sample created from the 10 samples analyzed. Bacterial growth results were assigned an index value of either 0, 1, 2, 3, or 4 based on reported growth, with 0 representing a negative result and values 1, 2, 3, or 4 a few, low, moderate, or high positive result, respectively. Growth values were reported as individual bacteria, with each sample receiving an overall index sum. The data were analyzed using the GLIMMIX procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC) with the Tukey-Kramer adjustment using the assigned location zones as the levels with the response variables of total growth (sum of index values) and presence of bacteria typical used to indicate fecal matter is present (fecal indicators). Across all facilities, zones 2, 3, 4, 6, and 7 had the similar reported mean growth values ($P \leq 0.05$) assigned, ranging from a mean index score of 18.1 for zones 2 and 3 to a mean index value of 21.4 for zone 7. Zones 1, 2, 3, 4, and 6 had similar mean growth values ($P \leq 0.05$). Zone 5 had the second-to-lowest demonstrated growth of all sample groups ($P > 0.05$), with the group of samples with the lowest growth index value being the raw ingredient and finished feed samples. There was evidence of moderate correlation noted ($r = 0.463$, $P \leq 0.0001$) between the zone and presence of EBAC, but no evidence of correlation ($r = 0.028$, $P > 0.05$) between zone a presence of fecal indicator bacteria. There was significant evidence of a weak correlation ($r = 0.201$, $P \leq 0.0001$) between EBAC presence and site.

Introduction: Environmental monitoring has been commonly used in food and other facilities manufacturing end-consumer products for years[1,2], and has gained traction as a method to determine the presence of pathogens that typically indicate fecal presence (fecal indicators)[3]. In addition to facilities producing direct-to-consumer goods, some healthcare systems have used environmental monitoring of both virus and bacteria to determine hygiene and biosecurity risk, including bacteria strains known to be resistant to antibiotics[4]. A correlation between the presence of Salmonella spp. and Enterobacteriaceae within feed mills has been demonstrated[5], but little information is available on how the presence of Enterobacteriaceae correlates with viral pathogen presence, especially on farms or in feed mills. The potential use of environmental monitoring of viral pathogens within a farm environment has seen an increase in popularity with the growing pressure placed on production systems from diseases like porcine epidemic diarrhea virus (PEDV), senecavirus A (SVA), and rotavirus. Environmental swabs have been shown to be effective when detecting viruses within feed manufacturing environments[6] and with on-farm use for swine operations. The ability for PEDV to be transmitted via contaminated feed ingredients and for contaminated feed to produce animal illness within research settings[7,8], as well as the epidemiological evidence to support a historical animal feed transmission within North America[9,10] has

brought increased levels of scrutiny on mills supplying feed to swine operations. The purpose of this study was to identify Enterobacteriaceae presence in the feed manufacturing facilities of a multi-farm system experiencing a viral outbreak as a method of identifying biosecurity gaps.

Objectives:
The purpose of this study was to identify Enterobacteriaceae presence in the feed manufacturing facilities of a multi-farm system experiencing a viral outbreak as a method of identifying biosecurity gaps.

Materials & Methods:
Swabbing method and location
Three separate feed manufacturing facilities (Sites 1-3) were evaluated and sampled for this study, with a biosecurity evaluation and audit (posted at ksuswine.org) performed during each visit. Each mill offered its own biosecurity challenges, either with the normal operating procedures or required tasks to be performed within facility limitations.

A total of 573 samples were taken over the course of four days, with 381 of those samples consisting of feed ingredient or finished feed, and the remaining 192 samples environmental swabs, collected across the 4 sites.
Feed ingredient and finished feed samples were collected using single-use plastic tubs. For each separate item, 10 individual samples were collected initially. For bulk-storage products, samples were either drop-collected or grabbed at multiple times while being conveyed. For bagged products, samples were obtained from each of 10 different bags onsite. Each sample was kept separate for individual analysis, with an additional blended composite sample created from the 10 samples analyzed.

For the environmental samples, two different collection methods were used. Cotton gauze swabs were utilized for areas within the mill that had easy access for swab collection. The gauze swabs were collected by swabbing a surface area of approximately 20cm x 20cm with a 10cm x 10cm cotton gauze square soaked in 5 ml of phosphate buffered saline (PBS) with a pH of 7.2. For areas without easy access, such as the interior of storage bins or truck trailers, a paint roller was utilized, as described by Dee et al., 201410. Locations did vary based on each individual site, but within the 3 feed manufacturing facilities (Sites 1-3), similar locations were chosen. Each swab was assigned one of four zones, including direct feed or ingredient contact surfaces (Zone 1), close proximity (within 1m) non-contact surfaces (Zone 2), non-contact surfaces without close proximity (>1 m of separation) (Zone 3), and transient surfaces, such as moveable tools, employees, and non-feed or ingredient delivery vehicles (Zone 4). Swabs taken from the fourth facility, the multiplier farm, were assigned zones based on proximity to pigs. This included direct feed-contact surfaces (Zone 5), direct pig-contact surfaces (Zone 6) including pen flooring, pen walls, feeders, and waterers (pig contact), and non-pig contact surfaces (Zone 7) including employee walkways, work areas, feed storage, and fans (non-pig contact).

Sample preparation and analysis
Feed and ingredient samples were collected individual. For each product, a composite sample was created by dividing and blending approximately 25 g from each individual sample. All product samples were stored at 4˚C until shipped. Cotton gauze

environmental swabs submitted for testing were initially prepared by adding 5 ml of PBS to a cotton gauze square in a conical tube prior to collection. After samples were collected, 20 ml of additional PBS were added. Swabs were kept at 4˚C until shipped. The paint rollers used for sample collection were placed into large zipper-seal plastic bags immediately after use. To prepare them for shipment, 200 ml of 7.2 pH PBS was added to each roller. The sample was then agitated and allowed to set for 1 hour. 10 ml of the PBS was removed from each sample and stored at 4˚C until shipped. After collection, samples were store and shipped on dry ice to the Iowa State Veterinary Diagnostic Laboratory. Samples were cultured on MacConkey agar, and the three types of bacteria with largest growth for each sample were identified and reported by assigning a growth index value.

Statistical analysis

Bacterial growth results were assigned an index value of either 0, 1, 2, 3, or 4 based on reported growth, with 0 representing a negative result and values 1, 2, 3, or 4 a few, low, moderate, or high positive result, respectively. Growth values were reported as individual bacteria, with each sample receiving an overall index sum. The data were analyzed using the GLIMMIX procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC) with the Tukey-Kramer adjustment using the assigned location zones as the levels with the response variables of total growth (sum of index values) and presence of bacteria typical used to indicate fecal matter is present (fecal indicators). Treatment means were separated using pairwise comparisons of means performed using the DIFFS option from the LSMEANS statement of SAS. Results were considered significant at $P \leq 0.05$. Data were also analyzed with the CORR procedure of SAS, with the variables including site, zone, and presence of fecal indicator bacteria.

Results & Discussion:

The U.S. feed industry is not designed for these types of cleaning and disinfection processes, so the primary focus must be on keeping pathogenic viruses out of feed mills, particularly because feed mills are a central point of cross-traffic among multiple farms or sites. Figure 1 demonstrates just a snapshot of the normal traffic flow of feed mills. Depending on mill size, it can receive dozens of ingredient delivery vehicles daily. The mill mixes ingredients together and distributes them to dozens of farms. The swine and poultry industries have developed highly effective biosecurity procedures that prevent people or transport vehicles from serving as fomites for viral. These include protocols for changing shoes, clothing, and/or showering and dynamic biosecurity pyramids for transport vehicles. Many modern swine production systems implement biocontainment practices similar to BSL2 or 3 laboratories but on a much larger scale. However, these same procedures have not been implemented at feed mills and with personnel or feed trucks.

Maps of EBAC levels per facility are shown in Figures 2, 3, and 4. Audit scores for each facility were 83%, 67%, and 42% for Site 1, 2, and 3, respectively. Site 1 utilized locked exterior doors, required employees to change clothes and shoes prior to entry, and had handwashing stations located inside the doorway. The scale was located within a fenced perimeter, and was used to weigh company-owned pigs occasionally. In Site 1, the scale, receiving pit, finished feed bin, and finished feed truck were the only feed-contact surfaces with detected EBAC. At Site 2, exterior doors were not locked and handwashing stations not used except for restroom purposes, but employees changed clothes and shoes prior to entry. There was no perimeter fence and the scale was routinely used to weigh animals. There was a moderate quantity of EBAC detected in all feed contact

surfaces tested, with high levels on the floor of the manufacturing area. At Site 3, exterior doors were not locked and handwashing stations not used except for restroom purposes, but employees changed clothes and shoes prior to entry. There was no perimeter fence and the scale was routinely used to weigh company-owned animals, as well as those from other within the region. While it was difficult to obtain samples from feed contact surfaces in Site 3, those collected all had high levels of EBAC.

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Clearly, compliance with biosecurity protocols had a substantial impact of Enterobacteriaceae prevalence and distribution throughout the feed mill. As facilities begin to transition biosecurity from the farm to the feed mill, using environmental monitoring to evaluate risk for biosecurity gaps, as well as success in their mitigation, will be useful and necessary.