Evaluation of a staged loading procedure for the load-out of market pigs to prevent the transfer of swine pathogen-contaminated particles from livestock trailers to the barn

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Industry Summary:

The swine industry has focused much of its efforts on improving biosecurity in breeding herds; while little attention had been paid in wean-to-finish growing sites. One risk event that has the potential to introduce virus into grow-finish pigs is load-out during marketing. In order for the remaining pigs in the group to become infected during load-out, viral contamination must be transferred from the contaminated livestock trailer, driver or other carrying agents to the pigs in the barn. Unfortunately, little research has been done to assess how frequently this occurs or to assess alternative biosecurity measures to reduce the frequency.

The objective of this study was to evaluate if implementing a staged loading procedure when loading out market pigs is effective at preventing transfer of swine pathogen contaminated particle form livestock trailers to the barn using the fluorescent powder (Glo Germ). The study compared a conventional method of loading and a staged loading procedure.

In standard loading protocols, there is usually only one line of separation implemented between the livestock trailer and the end of the load-out chute, in which the load-out crew members cannot cross over into the livestock trailer and the driver cannot cross over onto the chute. In a staged loading protocol, a second line of separation is implemented in addition to the first line of separation within the standard loading protocol. One member from the load-out crew is stationed between the two lines of separation in which he or she cannot cross onto the livestock trailer or cross the second line of separation into the center alleyway of the barn. The remaining load-out crew members within the barn cannot cross the second line of separation into the load-out alleyway or chute. There was 10 replicates per loading procedure.

216 g of dry Glo germ was mixed with approximately 0.5L of OB gel and 0.25 kg of dry wood chips in a large plastic bag and spread evenly on the floor of the livestock trailer just inside the roll-up door that opens to the chute. The second line of separation was also determined within every replicate and appropriately marked within the replicates that were performing the staged loading protocol. The load-out was observed and when the load-out was completed, Glo germ contamination was evaluated using a 120 x 55 cm2 grid that was divided into 264 5x5cm2 squares at 8 different measuring points within the chute after the first line of separation, two within load-out alleyway before the second line of separation, and five within the center alleyway.

Four out of the five measuring points in the center alleyway of the barn, had a level of contamination that measured significantly lower ($p<0.05$) for the staged loading protocol compared to the conventional loading protocol. The difference at the fifth measuring point in the center alleyway of the barn was nearly significant ($p=0.0573$). The level of contamination measured at all other measuring points, in the chute and loadout alleyway, were not statistically significant between the two study groups ($P>0.05$).

Scientific Abstract:

Objectives:
The objective of this study was to evaluate if implementing a staged loading procedure when loading out market pigs is effective at preventing transfer of swine pathogen contaminated particle form livestock trailers to the barn using the fluorescent powder (Glo Germ). The study compared a standard method of loading and a staged loading protocol.

Material and Methods:
Glo germ was used to asses a stage loading protocol when loading out market pigs by simulating contamination potentially carried by load-out crew members from the livestock trailer and chute back into to the barn. Load-out crew members either followed the standard load out protocol or the staged load out protocol. The standard load out protocol had only line of separation implemented between the livestock trailer and the end of the load-out chute, in which the load-out crew members cannot cross over into the livestock trailer and the driver cannot cross over onto the chute. The staged load-out protocol has a second line of separation in addition to the first line of separation within the standard loading protocol. One member is station between to two lines of separation in which he or she cannot cross onto the livestock trailer or passed the second line of separation into the center alleyway of the barn. The remaining load-out crew members within the barn cannot cross the second line of separation into the load-out alleyway or chute. There was 10 replicates per loading protocol. The Glo germ contamination was evaluated using a grid at eight different sampling points including within the chute after the first line of separation, two within the load-out alleyway before the second line of separation, and five within in the center alleyway, after the second line of separation.

Results:
Four out of the five measuring points in the center alleyway of the barn, had a level of contamination that measured significantly lower ($p<0.05$) for the staged loading protocol compared to the conventional loading protocol. The difference at the fifth measuring point in the center alleyway of the barn was nearly significant ($p=0.0573$). The level of contamination measured at all other measuring points, in the chute and loadout alleyway, were not statistically significant between the two study groups ($P>0.05$).The staged loading procedure completely eliminated contamination within the center alley measurements in one replicate, but did not completely eliminate contamination in all other replicates.

Introduction

The Swine Industry has focused much of its efforts in improving biosecurity in its breeding herds; while little attention had been paid in wean to finish growing sites. It has been estimated that 55% of groups of growing pigs that are negative for porcine reproductive and respiratory disease virus (PRRSV) at placement are positive at marketing, suggesting that PRRSV was introduced sometime during the growing period causing economic losses of approximately $2.29 per pig placed due to higher mortality and slower growth.[1] Although information on how frequently groups of growing pigs are infected with porcine epidemic diarrhea virus (PEDV) is not available in the literature, lateral introduction of the virus in growing pigs adversely affects average daily gain (ADG), average daily feed intake (ADFI) and

reduces growth.[2]. In one swine production system, the introduction of PEDV during late finishing reportedly reduced ADG by 21.4%.[3] Additionally, when growing pigs become infected, they serve as a source of virus that may increase the incidence of outbreaks in swine breeding herds, where economic consequences can be much larger. Data from the Swine Disease Reporting System demonstrates that significant increased detection of PRRSv in breeding herds are typically preceded by increased detection in growing-finishing pigs, supporting the hypothesis that the growing pig population is a major source of virus in the swine industry.[4]

One risk event that has the potential to introduce virus into grow-finish pigs is marketing. Typically, in the United States, groups of pigs are marketed over several weeks creating the opportunity for pigs still on feed to become infected after the first or interim load-outs are taken from the barn. The pigs remaining in the group are then subject to the production losses and become a source of virus for other swine farms. It has been demonstrated that livestock trailers can serve as a source of transmission for PRRSV and PEDV.[5,6]

For pigs remaining on feed to become infected during a marketing event, a series of failures is required. First, the livestock trailer, driver, truck or other carrying agent associated with the marketing event is contaminated with live infectious virus. Second, there is a failure to mitigate that contamination. Third, the virus is transferred from the contaminated livestock trailer or other contaminated carrying agent to the remaining pigs in the group as the pigs being marketed are loaded. Because swine slaughter plants receive animals from many sources daily, PRRSV and PEDV are likely present in the unloading area of swine slaughter plants. It has been demonstrated that the livestock trailers used to haul pigs to market frequently become contaminated with virus.[6] The driver as well as the cab of the truck may also serve as potential carrying agents for the viruses. Currently, many livestock trailers in the United States are not washed, disinfected or dried between loads of market pigs due to the lack of trailers, truck washes and other swine transport related infrastructure. If livestock trailers or other carrying agents associated with the marketing event are contaminated, it is unlikely that the contamination is mitigated. Therefore, when the livestock trailers, trucks and drivers returning directly from a swine slaughter plant enter growing pig sites to load market pigs; they remain contaminated with live infectious PRRSV, PEDV or both. For pigs, remaining in the group to become infected during loading, viral contamination must be transferred from the contaminated livestock trailer, driver or other carrying agents to the pigs in the barn. Unfortunately, little research has been done to assess how frequently this occurs or to assess alternative biosecurity measures to reduce the frequency.

In a previous study conducted by the investigators, the fluorescent powder (Glo Germ) (Glo Germ, Glo Germ Company, Moab, Utah) was used to evaluate if the addition of a bench entry system in a commercial swine facility with a shower reduced the likelihood of personnel introducing environmental contamination into a swine farm.[7] Glo Germ has the potential to show the transfer of environmental contamination from livestock trailer to barn. To determine if Glo Germ could be used to study the transfer of contamination from livestock trailers to barns, 216 grams of Glo germ mixed with 0.5 a liter of OB gel and 0.25 kg of woodchips were mixed together in a large zip loc plastic bag. Prior to loading, this mixture was spread evenly spread on the floor of the livestock trailer adjacent to the end of the load-out chute. After load-out, a fluorescent light was used to scan the load-out chute, load-out alleyway and center alleyway and

pens in the barn. Glo Germ could be found in the load-out chute, in the load-out alleyway, in the center alleyway, and in the first three pens adjacent to the center alleyway.

If viral contamination can be transferred from contaminated livestock trailers to the pigs in the barn during loading, what biosecurity measures may be implemented to reduce the frequency?

Objectives

The objective of this study was to evaluate if implementing a staged loading protocol when loading out market pigs is effective at preventing transfer of swine pathogen contaminated particle form livestock trailers to the barn using the fluorescent powder (Glo Germ). The study compared a standard protocol of loading and a staged loading protocol.

Materials and Methods

Study Facility Design

The study was conducted at 20 growing pig sites that were owned by a single production system. All study procedures were performed in accordance with the swine production and welfare policy of the production system. The twenty sites utilized in the study were sites that consisted of two attached barns and approximately 1200 pig spaces per barn. Inclusion criteria for the site layout included a loadout chute that was enclosed, immobile, and approximately 4 meters long and approximately 7.62 meters of center alleyway in the barn that loadout crew would walk after exiting the loadout area. Seventeen of the sites had a single loadout chute located in one of the barns, adjacent to a centrally located office. There was one loadout alleyway that led to the center alleyway in the barn. The load-out alleyway was enclosed on one side by a wall and on the other side by a 3 ft high solid cement side. The load-out alleyway was adjacent to a small holding pen with no pigs in it. One replicate in the conventional group had a single loadout chute at one end of both attached barns. Two of the sites, one replicate in staged and one replicated in conventional, had a loadout chute directly connected to a wide central hallway between both barns. The central hallway was enclosed by walls, and no holding pen was present. A standard double deck livestock trailer can hold approximately 170 market swine, therefore loads were only excluded if there were less than165 pigs loaded on a single trailer and if personnel stepped completely over the lines of separation established by the protocols more than twice.

Study Design

A control group (Conventional) and a treatment group (Staged) were compared in this study. Each treatment group had ten replicates. A replicate was defined as the final load of the scheduled loads for that site in a single day. The final load of the day was chosen to avoid delaying subsequent loads and disrupting market schedules while the measurements were taken. Each growing pig site was used for a single replicate to prevent the possibility of residual contamination from a previous replicate. Treatment groups were allocated to sites as shown in Table 2. This was based on the schedule of load-outs for the week and repeated replicates due to several discarded replicates.

Day of the Week	Conventional	Staged
Sunday	1	2
Monday	0	1
Tuesday	3	2
Wednesday	3	3
Thursday	2	1
Friday	1	1
Total:	10	10

Table 1: Displays the treatment group allocation per day of the week throughout the study.

For the Conventional group, a loading crew of 3 to 4 people loaded the pigs according to the production system's conventional loading protocol (Figure 1.A). Any member of the crew (Person 1) moved pigs from the center alleyway of the barn, through the loadout alleyway and up the chute. Person 1 was not allowed to cross the line of separation between the livestock trailer and the loadout. The driver was confined to the trailer or Zone A. The driver was not allowed to cross the line of separation between the chute and the back of the livestock trailer. The remaining members of the loading crew were restricted to Zone B and moved the pigs to be loaded from the pens down the center alleyway until they were transferred to Person 1 who moved the pigs the remainder of the way down the center alleyway, through the loadout alleyway and up the chute. The barn, loadout alleyway and chute were all part of the same zone (Zone B), and any member of the loadout crew could move freely within the zone.

For the Staged group, there were two lines of separation (Figure 1.B). The first line of separation was between the livestock trailer (Zone A) and the loadout chute in the loadout area (Zone B). The driver had to remain in the trailer. A single member of the loadout crew (Person 1) was designated to the loadout, or Zone B. The second line of separation was approximately between the loadout alleyway (Zone B) and the remainder of the barn (Zone C). Person 1 stayed in Zone B and the other members of the loadout crew (Persons 2 and 3) stayed in the barn, or Zone C. Person 1 was able to step into the buffer zone, which was considered part of the loadout (Zone B), to let pigs pass.

The same loadout crew, made up of four members, loaded 18 of the 20 replicates. They completed all ten of the Staged replicates and eight of the ten replicates for the Conventional replicates. Two other loadout crews were utilized for two Conventional replicates to accommodate loadout schedules. These two loadout crews had three members per crew. On day 1 of the study, a training session was conducted by the study investigators to train the loadout crew involved in the study how to perform the staged loading procedure. Two diagrams with instructions in English and Spanish on how to perform the Staged and Conventional loadout procedures were given to the loadout crew and loadout crew manager and explained in detail. Before each load-out for the study the load-crews were told which procedure they needed to perform, and reminded of the directions for that procedure.

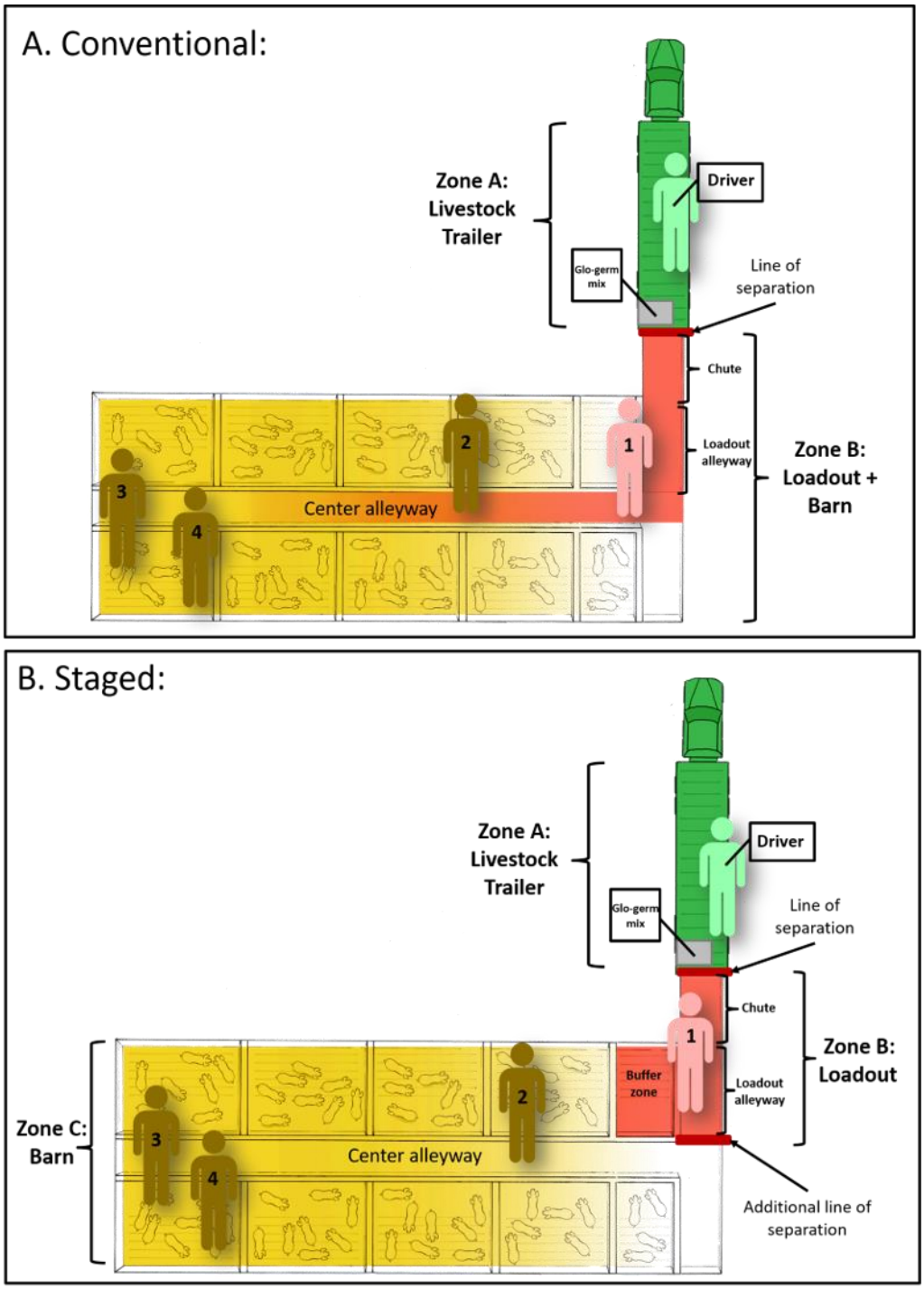

Figure 1: Description of the two treatment groups, 1) Conventional and 2) Staged. Fig. 1.A) For Conventional, there were two zones, Zone A: the livestock trailer and Zone B: the loadout area plus the barn. There was only one line of separation between the livestock trailer and loadout chute. All members of the loadout crew were free to move between the loadout chute, loadout alleyway and barn. Fig. 1.B) For Staged, there were three zones, ZoneA: the livestock trailer, Zone B: the loadout and Zone C: the barn. There were two lines of separation, with the additional line of separation located between the loadout area and the rest of the barn. Loadout crew member number 1 could not pass back into the barn, and loadout crew members 2, 3 and 4 could not pass into the loadout alleyway and chute.

Study Materials

Fluorescent Powder (Glo Germ):

Glo germ was used to visualize contamination from the livestock trailer to the barn. Glo Germ is a fluorescent powder that simulates the behavior of germs in fomites and is similar to size of bacteria, approximately 1 to 5 microns or less.[7] The powder appears white under natural light and when the powder is exposed by a U.V light it will fluoresce.

Measurement Grid:

One grid was constructed to measure the level of contamination (Figure 2). The grid was 120 x 55 cm^2 and divided into 264, 5 by 5cm^2 squares. The grid was made out of PVC pipes, metal eyelets and flat plastic string. The grids were coated with a fluorescent paint that was visible under UV light but a different fluorescent color than that of the Glo germ.

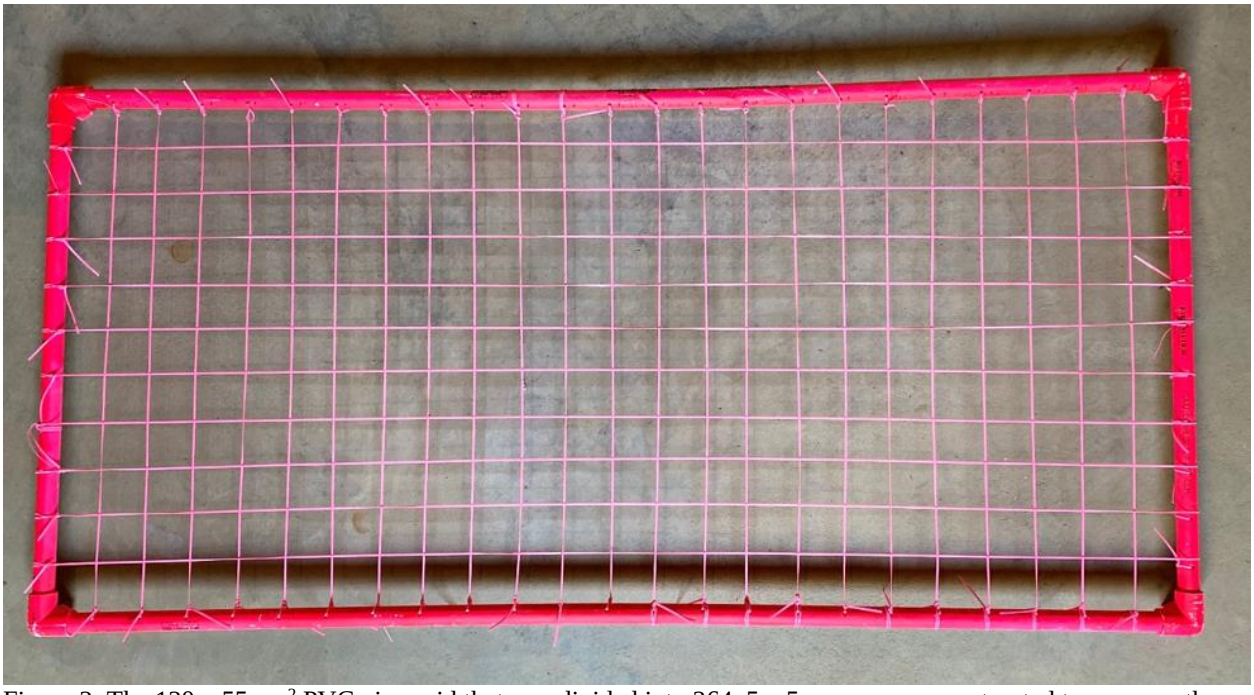

Figure 2: The 120 x 55 cm^2 PVC pipe grid that was divided into 264, 5 x 5 cm squares constructed to measure the level of contamination at 8 different measuring points after each load-out.

Study Procedures

Immediately prior to each scheduled site visit, 216 g of dry fluorescent powder was mixed with approximately 0.5L of OB gel and 0.25 kg of dry wood chips in a large plastic zip loc bag. When arriving at each site, just before the last schedule load-out for the evening, the fluorescent powder mixture was spread evenly on the floor of the livestock trailer just inside the

roll-up door that opens to the load-out chute. On the nights Staged replicates were performed, the location of the second line of separation was determined and marked with (spray paint) based on design of the barn. On the nights where the Standard replicates were performed, the location of the second line of separation was determined prior to load-out but not marked. The second line of separation was typically at the end of the load-out alley in the barn. When there was not a load-out alley in the barn, it was at the end of the central hallway where the barn and hallway met. If there was not an appropriate buffer area for Person 1 of the load-out crew in the Staged group to go while pigs passed, the second line of separation was extended approximately 2 feet into the center alleyway where Person 1 could establish a buffer zone.

After the Glo germ mixture was spread evenly on the back of the livestock trailer and the second line of separation was determined and/or marked, the load-out procedure was observed. Violations were recorded and deemed as minor, major or necessary violations. A minor violation would include a loadout tool crossing the line of separation or the partial crossing of the line of separation, such as half a boot. A major violation would include a person walking completely across the lines of separation. A necessary violation would include an animal going down on the load-out chute, trailer or halfway between the load-out chute and trailer, and a member of the load-out crew stepping completely over the line of separation or the driver needing to step completely over the line of separation, to help the animal. After the loadout was complete, Person 1 put on plastic boots by elevating their feet while standing in the loadout area and then stepping back into the barn. They were to avoid stepping on the floor of the loadout area once the plastic boots were on and return to the office to avoid cross-contamination after the second line of separation. After the load-out was complete, the load-out member that was stationed to the load-out chute placed on plastic boots to exit the barn to avoid contamination after the second line of separation. When the load-out crew exited the barn, the measurement grid was placed at 8 different measurement locations shown in Figure 3. The measurements included A. One foot in front of the end of the load-out chute adjacent to the trailer. B. One foot in front of the beginning of the load-out chute. C. One foot behind the second line of separation within the load-out alleyway. D1. Directly in front of the line of separation in the center alleyway. D2. In the center alleyway 3 feet after the previous measurement. D3. In the center alleyway 3 feet after the previous measurement. D4. In the center alleyway 3 feet after the previous measurement. D5. In the center alleyway 3 feet after the previous measurement.

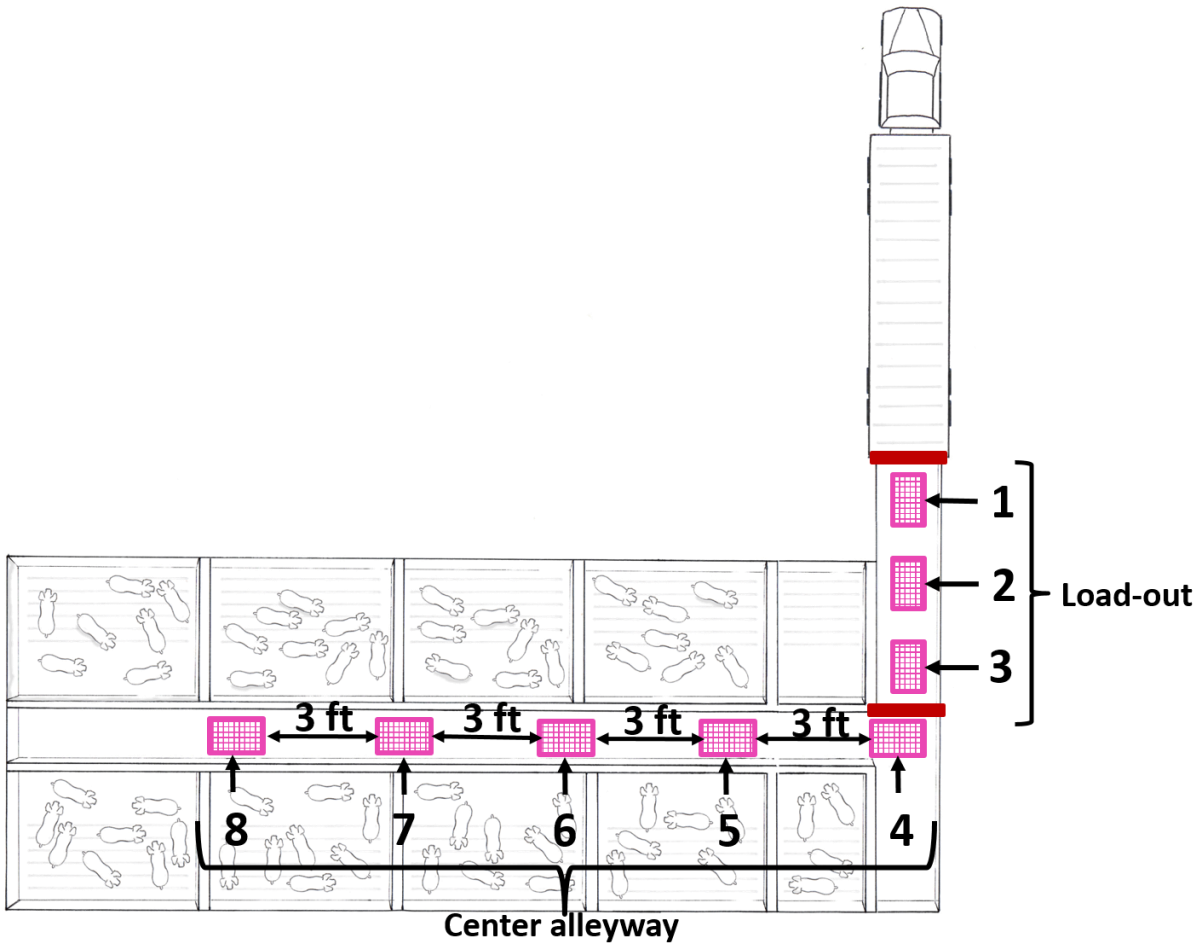

Figure 3: The measurement locations are displayed here. Measurements 1-3 were within the load-out. Measurements 4-8 were in the center alley, approximately 3 ft apart. The measurements included: 1. One foot in front of the end of the loadout chute adjacent to the trailer. 2. One foot in front of the beginning of the loadout chute. 3. One foot behind the second line of separation within the loadout area. 4. Directly in front of the line of separation in the center alleyway. 5. In the center alleyway 3 feet after the previous measurement. 6. In the center alleyway 3 feet after the previous measurement. 7. In the center alleyway 3 feet after the previous measurement. 8. In the center alleyway 3 feet after the previous measurement.

Statistical Analysis

All data was analyzed using R version 3.5.1. Contamination at each sampling point was compared between the Staged and Standard groups using a two-sided Wilcoxon rank sum test when normality conditions were not satisfied. Normality was checked using the Shapiro-Wilk test. Significance was set at the level of $P < 0.05$.

Results

Three replicates were discarded and repeated. One because the number of animals loaded onto the truck was significantly less than 165, one because there were over two major violations

from the staged loading protocol and one because the measurement grid did not fit in the loadout alleyway and an accurate measurement could not be obtained.

Data was successfully captured for all treatment replicates. The distribution of contamination at each measurement location for the two treatment groups is shown in Figure 4. The number of contaminated squares at measurement locations 4, 6, 7 and 8 in the center alleyway of the barn were significantly lower for the Staged group compared to the Conventional group ($P<0.05$). While the number of contaminated squares at measurement location 5 in the center alleyway of the barn was lower for the Staged group, the difference was not statistically significant ($P=0.0573$). The contamination at all other measuring points were not statistically significant ($P\geq0.05$) between the Conventional and Staged groups.

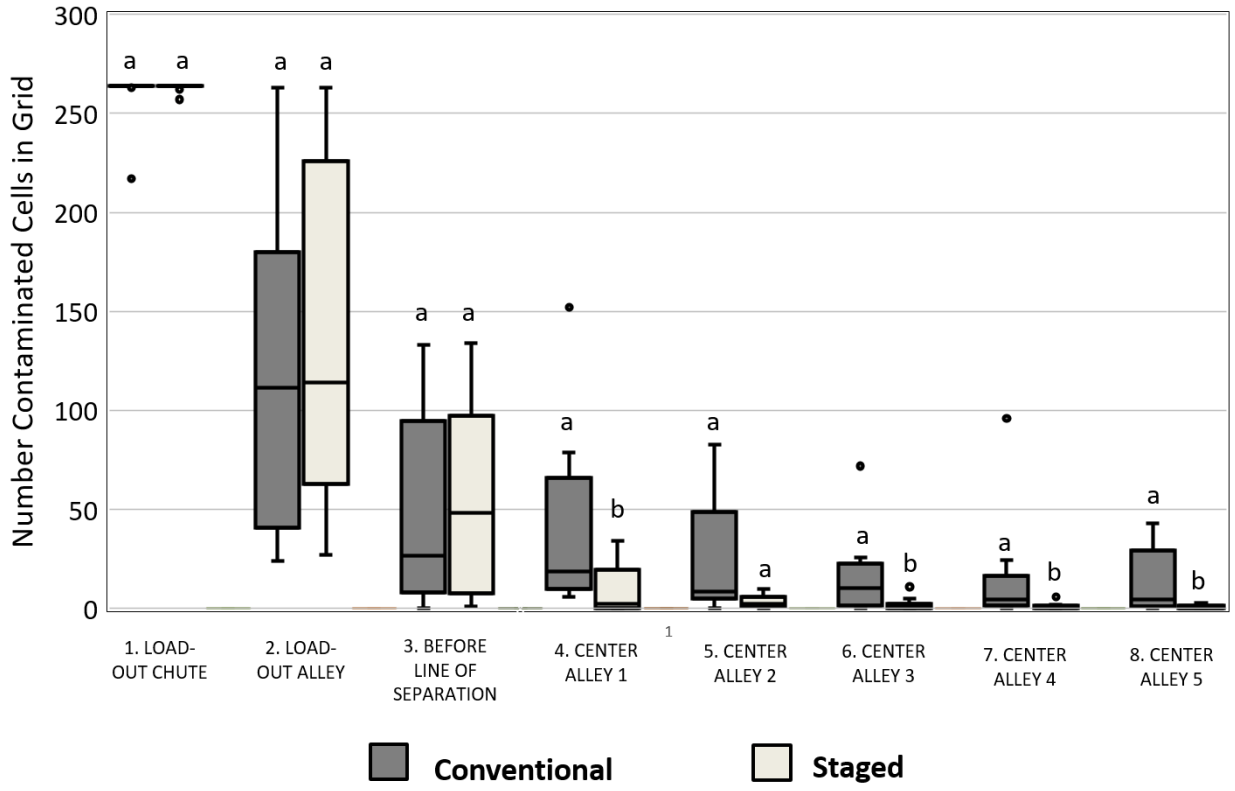

Figure 4: A box and whiskers plot displaying the distribution of the number of contaminated grid cells at each sampling point 1-8 for the Conventional and Staged groups. The whiskers represent the minimum and maximum values recorded for each point excluding outliers. Outliers are depicted by the dots. The upper boxes represent the medians of the 75th percentile and the lower boxes represent the medians of the 25th percentile. Different superscripts (a,b) within a sampling point indicates significant differences for Conventional and Staged groups. ($P<0.05$, two tailed Wilcoxon rank-sum test).

Discussion

The staged loading procedure significantly reduced the amount of contamination, simulated with dry fluorescent powder (Glo Germ), that was conveyed from the back of the trailer to the alleyway of the barn. The variation in measured contamination in the center alleyway of the barn for the Conventional group was also higher, with several outliers on the box

and whisker plots in Figure 5, compared to the Staged group. The large variation in the conventional loading procedures, suggests that the amount of contamination that is conveyed from a contaminated livestock trailer to the barn is inconsistent and likely depends on several factors, including how frequently the line of separation between the trailer and chute is violated. It was not expected that measurements 1-3, in the chute and loadout alleyway would be statistically significant from each other. The staged loading procedure will not affect how much contamination is conveyed from the trailer to the chute and loadout alleyway.

In both treatment groups, the measurements that were taken in the loadout chute consistently had a high level of contamination, with at least 75% of the squares in the grid contaminated. This demonstrates that the load crew member(s) that are walking on the chute, most likely were picking up contamination on their boots, sorting panels and other pig handling tools. The level of contamination on the chute was not only due to minor violations of the line of separation from personnel, but also from pigs lunging onto the trailer from the loadout chute and losing traction. To accelerate quickly or to go up an incline, pigs will lunge with their hind limbs. When they lose traction while lunging, bedding and contamination is ejected backwards onto the chute. This was observed in almost every loadout of the study and is a likely source of transfer of contamination from livestock trailer back into the loadout chute. Also, as more pigs are loaded, feces and urine accumulate on the loadout chute, allowing the wood shavings that are kicked from the trailer to stick to the boots of the loadout crew, allowing them to pick up contaminated particles and bring it back into the barn. An additional source of contamination in the loadout chute observed was from pigs turning around from the livestock trailer back onto the chute, which frequently happened throughout the study. Another factor observed during the study and contributing to the contamination of the loadout chute and loadout alleyway were minor, and sometimes necessary, violations of the procedure. Throughout the duration of the study, the loadout crew had several minor violations noted, and in one staged loading replicate, there was one major violation noted. . Some of these minor violations included a sorting panel crossing over the lines of separation, possibly picking up contamination and bringing it back into the barn. Approximately twice during the duration of the study, the driver exited the livestock trailer via the loadout chute when the loadout was complete.

In one Staged loading replicate, there was no contamination in the five measurements (measurements 4-8) in the center alleyway of the barn. However, in all other Staged loading replicates, the procedure did not completely eliminate contamination in the center alleyway of the barn. Contamination may have resulted from pigs turning around in the loadout alleyway or chute and crossing the second line of separation to return to the barn alleyway. Approximately 5 or more pigs turned around and crossed a line of separation during every loadout. Therefore, using appropriate gates or barriers may be warranted for staged loading to ensure that pigs cannot cross back over into the barn past the second line of separation. This may reduce the likelihood that the pigs would carry some contamination back into the barn from the livestock trailer. Other sources of contamination in the center alleyway of the barn were some minor violations, similar to those mentioned previously at the first line of separation. These minor violations, such as a sorting panel or boot crossing the second line of separation, were also observed.

Another challenge in the study was barn design. Some site designs were more complex and impacted the application of the staged loading procedure. Several of the study barns did not have a feasible buffer zone that was isolated from other pigs in the barn for the loadout crew member designated to the loadout area to step into while pigs were being moved into the loadout

area from the center alleyway of the barn. This did not interfere with the study, since a pen of pigs could be designated as the buffer zone therefore all measurements of contamination were taken in the center alleyway of the barn. However, this would defeat the purpose of using a staged loading procedure in practice. Ideally, a buffer zone would be adjacent to the loadout area, easy for Person 1 of the loadout crew to access and be isolated from other pigs in the barn. It would be beneficial for a buffer zone to be away from pigs in the barn so that cleaning and disinfection of the loadout area and buffer zone could be accomplished without contaminating the remaining pigs in the barn. Barn design must be considered when implementing a staged loading protocol.

There were several complications that took place during the study. Ideally, sites would have been randomly blocked by day of the week. However, due to the three replicates being discarded and the production systems loadout schedule for sites with the inclusions criteria, this was not feasible. More research may need to be done to see if day of the week has an impact on loading protocols.

Three loadout crews were used in this study, as mentioned previously. This is not believed to impact study results due to the fact that both load-out procedures (conventional and staged) were the same and all loadout crews were trained in the procedures performed. The main objective of this study was to compare the level of contamination within the center alleyway of the barn between to two treatment groups. During the conventional loadout procedure, when there were 4 crew members present, the member that runs pigs up the loadout chute usually walks back into the barn approximately 7.62 meters in the center alleyway, until the next group of pigs is brought to them. When there were 3 crew members, the member that runs the pigs up the loadout chute also walks back into the barn approximately 7.62 meters into the center alleyway, where the center alleyway measurements were taken. The only observable difference between the three member loadout crew and the four member loadout crew would be that the member running the pigs up the loadout chute may need to travel further than the 7.62 meters back into the center alleyway to receive the next group of pigs. Both crews (three vs four) covered the same area that was measured in the center alleyway.

The grid was a novel measurement approach first used in a study looking at the addition of a bench entry system to reduce the level of contamination.[7] As in the previous study, the grid was used to quantify the level of contamination at the measurement points within the barn. However, if any Glo Germ was observed in each 5x5 cm square, the square was counted as contaminated. The coverage within the square may have ranged from a small particle to complete coverage in the square.[7] A higher resolution grid would result in a more precise measurement of contamination.

Under the conditions of this study, staged loading reduced the amount of contamination conveyed from livestock trailers to the barn, but did not completely eliminate it. This study highlights the importance of additional layers of biosecurity. Adding layers of biosecurity can reduce the frequency that contamination is conveyed from the livestock trailer to the barn. When contamination crosses the first line of separation, the second line of separation serves as a back-up to reduce contamination transfer from the loadout chute to the center alleyway in the barn.

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