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Summary

During the spring of 2021, the Kansas State University Swine Early Wean Facility (SEW) experienced a notable increase in piglet morbidity and mortality. Piglet diarrhea was observed approximately 2 to 3 weeks post-weaning along with an increase in number of sudden mortalities. Necropsy samples were collected and confirmed for clinical diagnosis of *Escherichia coli* K88 infection by the Kansas State University Veterinary Diagnostic Laboratory. *E. coli* K88 can negatively impact performance of pigs and typically manifests as diarrhea, which can continue until death because of severe dehydration and metabolic acidosis or from terminal septicemia. Once present, *E. coli*, including *E. coli* K88, tends to persist in the environment unless vigorous efforts are successful at sanitation and disinfection. Therefore, the overall objective of this study was to determine the critical areas in need of improved disinfection at the nursery facility and to make recommendations based on environmental sampling results. The research team surveyed the most probable areas of contamination before sampling and identified six locations from which to collect environmental samples in each pen. These six locations, in addition to other common-use areas in the barn, were sampled using sponges and swabs from 10 pens at random both pre- and post-disinfection. After the completion of sampling, samples were enumerated using Sorbitol MacConkey Agar with cefixime and tellurite (CT-SMAC). *E. coli* was not detected from the common-use areas such as the water lines, office water faucets, and feed buckets. The dirtiest pen sample areas pre-disinfection included under rubber mats, inside and outside of waterers, and the floor slats. Disinfection significantly reduced ($P < 0.05$) contamination of the floor slats and the waterer (inside and outside). While the slats were initially among the dirtiest samples, after cleaning, a 6.5 log reduction was observed. Conversely, contamination on the feeder surface and lip of the feeder was not significantly reduced post-disinfection ($P > 0.05$). *E. coli* was recovered from every sample type post-sanitation. While the current cleaning process was successful in reducing bacterial contamination, these data suggest it could be further improved by using a more effective and thorough cleaning process, as some residual contamination remained. Recommendations might include the use of a stronger disinfectant with power washing, higher water pressure, and increased water temperatures, among others. Perhaps physical scrubbing in hard-to-reach locations, such as rubber mats and water cups might also be helpful.

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Introduction

Over the months of December 2020 through April 2021, there was a noticeable increase in mortality rate at the Kansas State University Swine Early Wean Facility (SEW). Following clinical diagnosis of *Escherichia coli* K88 infection by the Kansas State University Veterinary Diagnostic Laboratory, the research team determined that an evaluation of the facility disinfection procedures was warranted. This evaluation of disinfection procedures would take into consideration the high-use and high-risk areas where disinfection would be critical to reduce pathogen carryover from one animal group to the next. Disinfection procedures were evaluated through the comparable reductions of *E. coli* bacteria present on several environmental surfaces within pens both pre- and post-disinfection.

Enterotoxigenic *Escherichia coli* (ETEC) strains are frequent causes of piglet diarrhea during the preweaning and immediate postweaning periods. Among the different ETEC strains (K88-, K99-, or 987P-expressing strains), those expressing K88 fimbrial antigen are the most prevalent.² When isolated and cultured, most pathogenic strains form smooth to mucoid colonies on XLD or SMAC media; some are beta-hemolytic. Virulence factors include fimbria (pili), enterotoxins (exotoxins), endotoxins, and capsules. Fimbria are the small hair-like processes on the bacterial surface that allow attachment to specific receptors on the surface of mucosal enterocytes of the small intestine (colonization). Pathogenic strains also produce one or more enterotoxins, which are exotoxins elaborated locally in the small intestine that can have either local or systemic effects.³ These fimbriae mediate the adhesion of *E. coli* K88-expressing strains to the intestinal epithelial mucosa and to the mucus layer lining the small intestine. Thereafter, the organism elaborates one or two enterotoxins, heat-stable toxin and heat-labile toxin, which induce massive fluid and electrolyte secretion into the gut lumen. Antibiotics are routinely used in an attempt to control pathogens, but the organisms are becoming resistant to the more commonly used treatments, making antibiotic therapy unreliable.⁴

E. coli, typically, enterotoxigenic strains such as K-88, can cause substantial issues in pig production. If gilts farrow before they have developed antibodies to endemically present pathogenic *E. coli*, their colostrum and milk may not contain enough antibodies to protect their piglets. Also, as the nursing period progresses, piglets get less milk and the milk contains fewer antibodies. Chilling of piglets impairs intestinal motility and lowers resistance to infection. In recently weaned pigs, absence of milk antibodies and the different type of feed may contribute to outbreaks of this bacteria. Often, piglets can also contract the disease if it is present on or contaminating the sows' mammary glands.

² Sun, Y., & Kim, S. W. (2017). Intestinal challenge with enterotoxigenic *Escherichia coli* in pigs, and nutritional intervention to prevent postweaning diarrhea. *Animal nutrition (Zhongguo xu mu shou yi xue hui)*, 3(4), 322–330. <https://doi.org/10.1016/j.aninu.2017.10.001>.

³ *Colibacillosis (E. coli diarrhea)*. Iowa State University. (n.d.). <https://vetmed.iastate.edu/vdpam/FSVD/swine/index-diseases/colibacillosis>.

⁴ Mazzoni, M., Meriardi, G., Sarli, G., Trevisi, P., & Bosi, P. (2010). Effect of two doses of different zinc sources (Inorganic vs. Chelated form) on the epithelial proliferative activity and the apoptotic index of intestinal mucosa of early-weaned pigs orally challenged with *E. coli* K88. *Asian - Australasian Journal of Animal Sciences*, 23(6), 777+. <https://link.gale.com/apps/doc/A224990360/AONE?u=ksu&sid=AONE&xid=cf03ccc2>.

Symptoms of an *E. coli* K-88 infection may include the reduced absorption of electrolytes, water, and endogenous secretions from the lumen. The large intestine, sometimes also affected, is unable to absorb the resulting excess fluid and diarrhea. Damage to epithelial cells sometimes leads to septicemia. Diarrhea usually continues until death as a result from dehydration and metabolic acidosis or from terminal septicemia. The tell-tale signs shown are diarrhea that usually has an alkaline pH but varies in color. It may be clear and watery, especially in neonates, but may be white or yellow, and is influenced by type of ingesta and duration of the disease. Sick pigs occasionally vomit but vomiting is not as prominent as with transmissible gastroenteritis (TGE). The infection of K-88 remains a substantial problem, as on average, K-88 positive-receptor pigs have lower average daily gain (ADG), compared to the negative-receptor pigs.⁵

In general, pathogenic *E. coli* can survive in contaminated buildings and can infect successive groups of pigs. Once present, *E. coli* tends to persist unless vigorous efforts are undertaken to improve sanitary conditions and husbandry. The overall objective of this study was to 1) use *E. coli* populations as an indicator of cleanliness and disinfection efficacy, thereby determining the critical areas in need of additional sanitation at the SEW at Kansas State University; and 2) make recommendations based on the environmental samples collected.

Procedures

Experimental design

A total of 10 pens were randomly selected for environmental sampling. From each pen, 6 samples were collected before and after disinfection, with the same pens sampled for each sampling point. Barn 1 contained pens labeled 1 to 40 and Barn 2 contained the pens labeled 41 to 80. Pens used in this study were selected using the RAND function of Microsoft Excel (Microsoft, Redmond, WA). Environmental samples from common use areas (e.g., feed buckets) were also collected. Prior to loadout, oral fluid from rope samples were analyzed. The pens randomly selected for environmental sample collection were sampled both pre- and post-disinfection of the SEW (Figures 1 and 2).

Sampling plan

Prior to sampling, the research team surveyed the SEW to identify the most probable areas of contamination. A total of 6 sampling sites were identified and are described in Table 1. From each pen, these 6 environmental samples were collected, and a total of 10 pens were sampled (5 pens from each barn), for an overall total of 60 samples. Additional “common use” sampling locations (e.g., medicator pump) were selected based upon their potential for contact with, and subsequent contamination of, the entire swine population in a barn. Environmental sampling occurred as follows:

1. March 22, 2021: Immediately after loadout
2. March 26, 2021: Four days after cleaning and disinfection

Sampling procedures

Prior to sampling, facility-owned protective equipment and latex gloves were utilized to prevent any facility-to-facility contamination.

⁵ Jin, L. Z., Marquardt, R. R., & Zhao, X. (2000). A strain of *Enterococcus faecium* (18C23) inhibits adhesion of enterotoxigenic *Escherichia coli* K88 to porcine small intestine mucus. *Applied and environmental microbiology*, 66(10), 4200–4204. <https://doi.org/10.1128/aem.66.10.4200-4204.2000>

From each of the 10 pens, 1 oral fluid sample was collected prior to the pre-disinfection sampling period to quantify *E. coli* populations in the oral cavity of the pigs. Oral fluid samples were collected from ropes provided to each pen of pigs. The oral fluids were extracted from the rope by SEW personnel and provided in plastic tubes for analysis on March 15, 2021.

Table 1 summarizes all sample types collected from each pen. All samples in pens, both with cotton-tipped swabs and sponges, were collected using a back-and-forth motion to physically remove residue and/or bacterial contamination. Metal slats were first to be sampled, to avoid the potential risk of contamination from boots as personnel entered the pens. Prior to sampling, cotton-tipped swabs were wetted with Dey-Engley neutralizing buffer (DE; 3M, St. Paul, MN) and applied to the interior lip of feeders. Feeder lips were swabbed across the entire length of the feeder lip. After sampling, the cotton-tipped swabs were placed into sample tubes (MidSci, Valley Park, MO) containing 10 mL of DE. Similarly, the “community use” environmental samples collected from the medicator pump (filter and water lines) and water faucets were swabbed with a DE-wetted cotton-tipped swab and placed into sample tubes containing 10 mL of DE. Sponges pre-hydrated with 10 mL of DE were employed for all other sample areas. After completion of sampling, samples were transported to the Kansas State University Food Safety and Defense laboratory for processing.

Pre-disinfection, water and fecal samples were aseptically collected in a sterile sampling cup (VWR, Radnor, PA) or Whirl-Pak bag (Nasco, Madison, WI). Briefly, water was collected directly into a sterile sampling cup at the “community use” water faucet. Sterile sampling spoons were used to collect the fecal samples from the floor of random pens and placed directly into the sampling bag.

Disinfection process

After pre-disinfection samples were collected from barns, the normal disinfection and power washing procedures took place. This included separating feeders from pen walls, providing a coating of BarnStorm (Neogen, Lansing, MI), and power washing all areas in barns thoroughly, with cold water, roughly 50–60°F (10–16°C) with water pressure at 3000 PSI. After washing, barns were further disinfected with Synergize (Neogen, Lansing, MI) twice. Barns were allowed 1–2 days of drying time before post-disinfection samples were collected.

Environmental sample processing

Although sponge samples were pre-wetted with 10 mL of DE, the sponge samples collected for the pre-disinfection sampling point were dry from the sampling procedure and the organic matter captured on the sponge. For these reasons, a liquid sample was unable to be obtained during sampling processing and the original DE liquid was negligible. Therefore, sponge samples collected at the pre-disinfection sampling point were homogenized for 1 minute with 100 mL of 0.1% peptone water (PW; BD Difco, Sparks, MD) to rehydrate the sponge and provide an immediate 100-fold dilution of the large bacterial load. Subsequent serial dilutions were prepared in PW. Sponge samples collected post-disinfection retained their moisture, likely due to the absence of organic matter after the cleaning procedure. Post-disinfection sampling sponges were homogenized for 1 minute and the remaining DE fluid was used to prepare serial dilutions in PW. Swab samples in DE were vortexed and serially diluted in PW. Pre-

and post-disinfection samples were spread-plated in duplicate on Sorbitol MacConkey agar (SMAC; BD Difco, Sparks, MD) plates and incubated at 98°F (37°C) for 18–24 h. Following incubation, colonies with a pink or colorless to opaque appearance were counted and recorded as generic *E. coli* or presumptive *E. coli* O157, respectively. Samples that fell below the limit of detection for enumeration were enriched in tryptic soy broth (TSB; BD Difco, Sparks, MD) for detection. Briefly, samples were homogenized with 2× TSB (diluted by the original DE or PW sample to 1× TSB), incubated for 18–24 h at 98°F (37°C), streaked to SMAC, and then incubated at 98°F (37°C) for 18–24 h. Following incubation, plates without growth were recorded as “not detected”.

Water, fecal, and oral fluid sample processing

Each water sample was vortexed, a 1 mL aliquot was mixed in 9 mL of DE, serial dilutions were prepared in PW, and spread-plated on SMAC. From each fecal sample, 10 g of feces were homogenized in 90 mL of DE, serially diluted in PW, and spread-plated on SMAC. From each oral fluid sample, 1 mL of oral fluids was mixed with 9 mL of DE, serially diluted in PW, and spread-plated on SMAC. All SMAC plates were incubated and data recorded as previously described.

Statistical analysis

Generic *E. coli* and presumptive *E. coli* O157 counts were combined and reported as a single *E. coli* population plate count value for each sample that represented total contamination. These data were recorded and analyzed using GraphPad Prism 9 (La Jolla, CA). For each of the six environmental sample types, data were analyzed using a Wilcoxon matched-pairs signed rank test to compare pre- and post-disinfection *E. coli* populations. All environmental data are reported on a CFU/sample basis. Water samples and oral fluid samples are reported as CFU/mL, while fecal samples are reported as CFU/g, and the means with standard deviation of these data were calculated using Microsoft Excel (Redmond, WA).

Results and Discussion

Disinfection significantly reduced *E. coli* contamination on the floor slats ($P = 0.004$), inside of cup waterers ($P = 0.002$), and outside of cup waterers ($P = 0.002$). Conversely, disinfection efforts did not significantly reduce *E. coli* populations on the surface of the feeder ($P = 0.131$) or underside of the feeder lip ($P = 0.084$). *E. coli* persisted on every sampling site post-disinfection. *E. coli* populations ranged from 3.9 to 9.6 log CFU/sample pre-disinfection and declined to 1.0 to 4.2 log CFU/sample post-disinfection. The rubber mats under the waterers harbored the most contamination post-disinfection, with an average of 4.2 log CFU/sample of *E. coli* per pen. The metal floor slats were the least contaminated post-disinfection, with an average of 1 log CFU of *E. coli* per sample.

Contrary to our hypothesis that “community use” areas could be a potential source of *E. coli*, the medicator pump (filter, water lines, and water) and water faucets did not harbor *E. coli* when sampled during the pre-disinfection sampling period. Because these sites were clean prior to disinfection, they were not re-sampled post-disinfection. Similarly, the mean *E. coli* population for the two water samples collected during the pre-disinfection period was 0.2 log CFU/mL.

Oral fluids collected from each pen of pigs harbored *E. coli* at populations ranging from 4.8 to 6.9 logs, with an average of 6.4 log (± 0.7 SD) CFU/mL. The two fecal samples were contaminated with approximately 9 log CFU/g of *E. coli*.

The results of this case study demonstrate that current disinfection practices were not effective at eliminating contamination between groups of pigs. Several sites within pens were identified as harboring contamination post-disinfection, which suggests more focused cleaning and sanitation efforts are needed. Specific attention during cleaning should be given to feeders as well as areas under and around the mats in pens. Re-evaluating the disinfectant in use, including a different disinfectant after power washing, and using scrubbing brushes to reach obstructed places (e.g. under feeder lips and rubber mats) may also improve cleaning and sanitation efforts. Routinely testing for microbial contamination after cleaning and disinfecting to ensure that the cleaning process is effective may also be helpful.

The sampling points used in this study were collected based on probable harborage of *E. coli* bacteria; however, there are additional areas or surfaces within the barns which potentially warrant further observation. Ventilation, feed distribution, flies and other insects, and potential for transfer of organisms between barns should all be considered when evaluating best practices at the facility evaluated.

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Table 1. Pen sites and sampling methods used for environmental sampling at swine nursery facility

Sampling site ¹	Description	Sampling method
Feeder lip	Underside of curved lip – entire length	Cotton-tipped swab
Feeder surface	Front surface directly above feed opening	Sponge
Rubber mat	Underneath rubber mats located below waterer	Sponge
Slats	Top surface and in between slats of metal flooring	Sponge
Cup waterer	Inside surface	Sponge
Cup waterer	Outside surface	Sponge

¹Two water samples, two fecal samples, and other “community use” locations were sampled during the pre-disinfection period, and included the medicator pump (filter, water, and water lines) and water faucets.

Table 2. Pre- and post-disinfection *Escherichia coli* populations of 10 pens at swine nursery facility¹

Sampling location	Pre-disinfection		Post-disinfection		P-value
	Mean log CFU/sample	Standard deviation	Mean log CFU/sample	Standard deviation	Comparing pre- to post-disinfection
Feeder lip	5.0	2.4	2.5	1.6	0.084
Feeder surface	3.9	3.0	2.4	0.9	0.131
Rubber mat	8.3	1.6	4.2	1.0	0.004
Metal flooring (slats)	7.2	2.8	1.0	1.2	0.004
Cup waterer-inside	9.6	0.9	2.5	1.5	0.002
Cup waterer-outside	7.5	1.9	2.2	2.0	0.002

¹One sample from each location was collected from the same 10 pens pre- and post-disinfection.

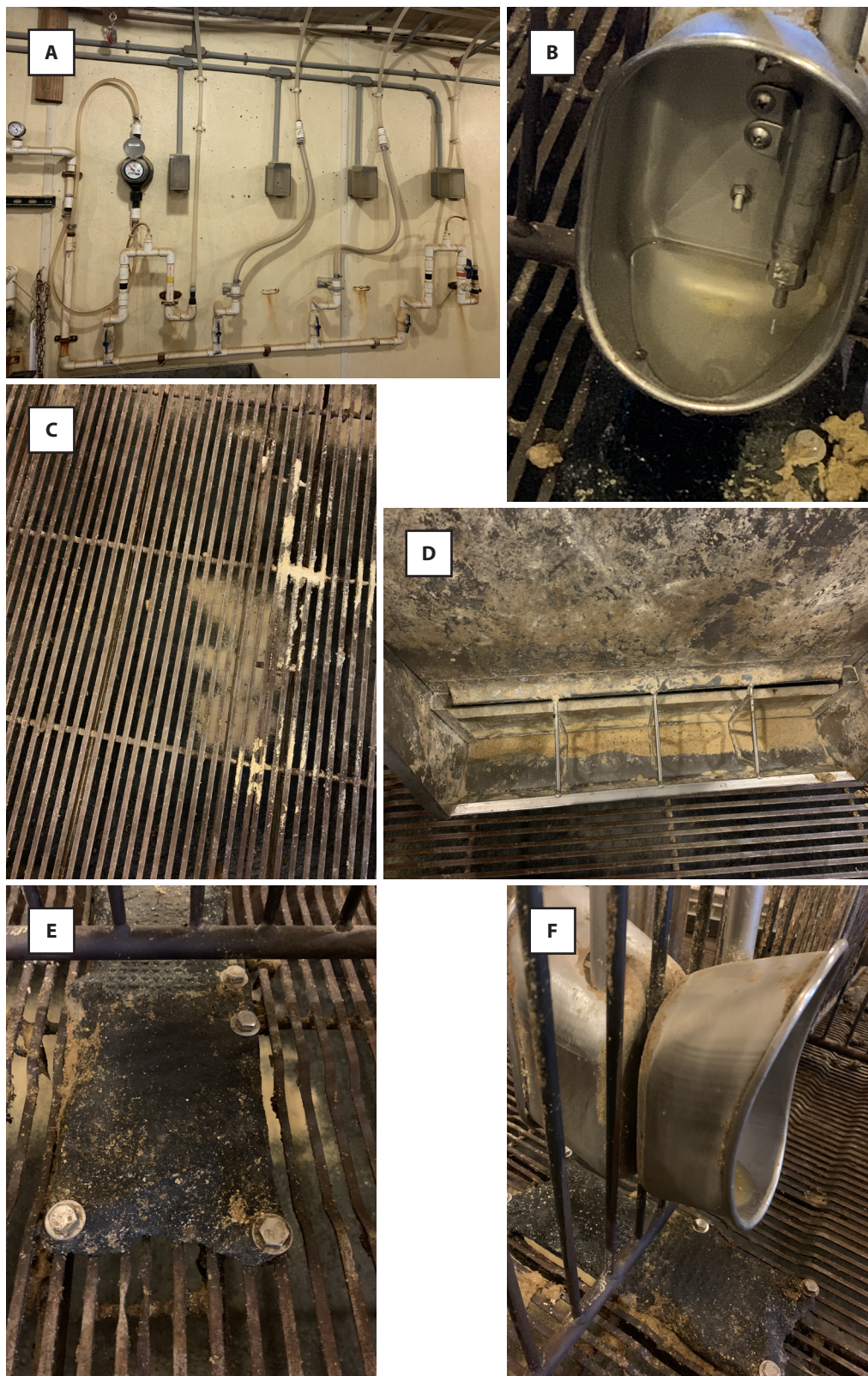


Figure 1 (A-F). Environmental samples collected at the SEW pre-disinfection.
A) Water lines in barns swabbed. B) Inside of waterers swabbed. C) Slats swabbed. D) Feeder lip and surface swabbed. E) Mats under waterers swabbed. F) Outside surface of water cup swabbed.

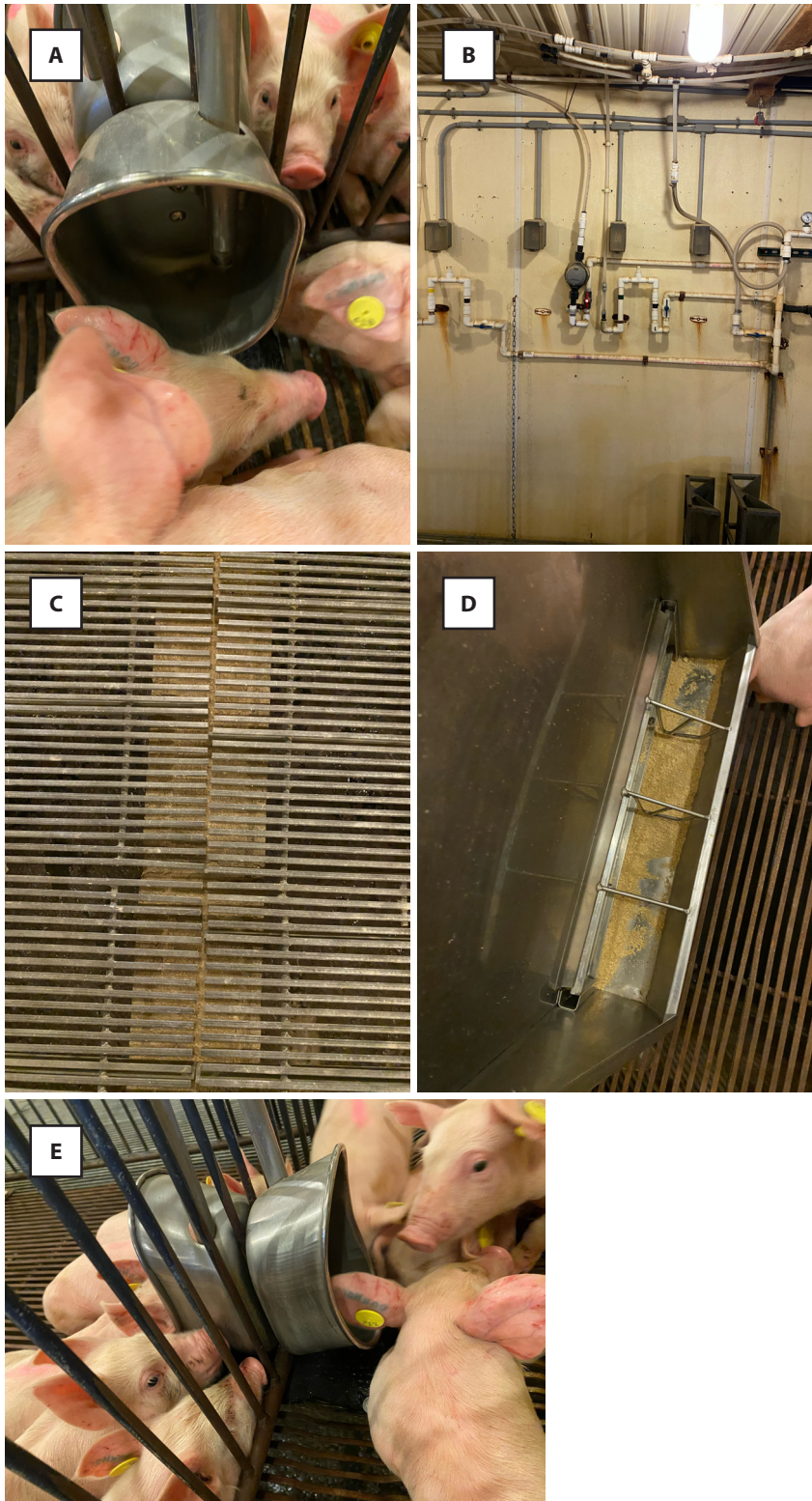


Figure 2 (A-E). Environmental samples post-cleaning. Although animals are shown in the photos, samples were collected *prior* to the arrival of new groups of pigs.

A) Clean inside of waterer. B) Clean water lines in barns. C) Clean slats. D) Clean feeder/feeder lip. E) Cleaned outside of waterer.

*Picture files separately included of environmental samples collected at the SEW pre-disinfection as well as environmental samples post-cleaning. Although animals are shown in the post-cleaning photos, samples were collected *prior* to the arrival of new groups of pigs.