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Preventing *Salmonella* infection in pigs with offsite weaning

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Summary

Purpose: To evaluate a strategy to prevent *Salmonella* infection in pigs from a herd with an ongoing clinical problem with *S. choleraesuis* by weaning pigs offsite.

Methods: Fifty-six 10- to 16-day-old pigs were purchased from a herd that had an ongoing problem with *S. choleraesuis*, and were moved to an offsite facility. On the day the pigs were purchased (day 0), milk and rectal swab samples were collected from sows in the herd of origin. On days 1, 43, 83, and 109 of the study, rectal and oropharyngeal swabs were collected for *Salmonella* spp. culture and sera were collected for serologic testing for *Salmonella* antibodies from all offsite pigs. To determine whether there was continuing on-farm transmission of *Salmonella* spp., fecal swabs for culture and sera for ELISA testing were collected from 15 age-matched pigs that remained on the farm of origin. These age-matched pigs were moved on day 61 of the study to an outdoor grow-finish lot, where they were raised to slaughter. Environmental samples were collected from the outdoor growing-finishing pen (from bowl waterers and mud holes) for culture.

Results: In the offsite pigs, no *Salmonella* spp. were isolated and there was no serological evidence of *Salmonella* exposure. On the farm, *Salmonella* spp. were isolated from rectal swabs from seven of the 15 (46.6%) onsite, age-matched pigs, from 11 (including one sample from which *S. cholerasuis* was isolated) of the 24 (45.9%) environmental samples, and from one of 24 (4.2%) rectal swabs from sows. Between days 61 and 99 of the study, mean anti-*Salmonella* titers for onsite pigs increased, indicating ongoing exposure to *Salmonella* spp.

Implications: Offsite weaning at 10–16 days of age prevented *Salmonella* spp. infection in grow-finish pigs moved to a clean environment.

Keywords: swine, *Salmonella*, environment

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Salmonellosis is an economically important disease of pigs. Mortality, weight loss, and poor growth due to clinical disease—primarily caused by *Salmonella choleraesuis*—cost the swine industry millions of dollars annually.[1,2] Salmonellosis is also an important foodborne illness in humans.[3] Zoonotic *Salmonella* spp. are commonly isolated from pigs at slaughter and contaminated pork products are considered to be a source of human infection.[1,3,4] Consumer fear of salmonellosis can potentially reduce sales of pork. Management strategies that can prevent or reduce *Salmonella* infection would be of major economic value to the pork industry.

Moving growing-finishing pigs to a site removed from the breeding herd is a common strategy used to improve health and increase growth.[5–8] Although not all trials were successful, Fedorka-Cray, et al., raised and maintained pigs from which *Salmonella* spp. could not be cultured through 6 weeks of age by weaning and finishing them offsite.[7] Fedorka-Cray, et al., obtained the pigs in their study at 10–21 days of age from farms that reportedly had past histories of salmonellosis, but which had not had any clinical problems during the past year. Our objective in the present study was to evaluate a protocol for preventing *Salmonella* infection in 10- to 16-day-old pigs, obtained from a herd with severe clinical salmonellosis, by weaning them to an uncontaminated environment.

Materials and methods

Herd history

Pigs used in this trial were from a 450- to 500-sow commercial herd that had been experiencing 24% death loss during the grow-finish phase of production for 9 months. Pigs in this herd were moved at about 22.7 kg (50 lb) from indoor nursery pens to outdoor grow-finish pens, where they were raised to market weight. Typically, 1–2 weeks after being placed in outdoor pens, several pigs were found dead and other pigs were depressed and displayed signs of respiratory difficulty. Within a few days, many pigs developed diarrhea, and some of these pigs died after several days to weeks of protracted diarrhea and weight loss.

On several occasions (Table 1), the herd owners had submitted pigs to the Kansas State University (KSU) Veterinary Diagnostic Laboratory, and each time *S. choleraesuis* was isolated from visceral organs.

Sows were vaccinated with an autogenous *S. choleraesuis* bacterin 5 and 2 weeks prior to farrowing. Two weeks after we purchased the pigs used in this study, the herd owners began vaccinating all piglets at weaning with modified-live *S. choleraesuis* vaccine (SC-54, NOBL Laboratories, Sioux Center, Iowa).

Table 1

Diagnostic data from herd of origin

Date submitted	Number of pigs submitted	Tissues submitted	Number of tissues from which S. choleraesuis was isolated
28 April 1993	3	livers	3 of 3
8 December 1993	8 (4 live, 4 dead)	lung, liver, spleen, kidney	at least one organ in all 8
3 January 1994	3	lung, liver, spleen, kidney	from all four organs in 2 of 3
11 April 1994	2	lung, liver, spleen	2 of 2 (all organs)
21 April 1994	3	lung, liver, heart	3 of 3 (lung, liver) and from heart valve of one pig with valvular endocarditis

Experimental design

Offsite pigs

Fifty-six 10- to 16-day-old piglets were purchased from the commercial herd for this study and transported to KSU (Figure 1). On the day after purchase (day 1 of the study), the pigs were randomly allocated into one of two experimental groups:

- piglets (n = 28) that received daily intramuscular injections of 0.3 mL (15 mg) ceftiofur (Naxcel®, Upjohn Company, Kalamazoo, Michigan) on days 1–5; or
- piglets (n = 28) that received no parenteral antibacterial agents throughout the trial.

The piglets were housed according to treatment in groups of seven in elevated plastic tubs. For the first 43 days of the trial, all pigs received a three-phase early weaning diet that contained 50 g carbadox (for its growth promotion effects) per ton of feed.[6]

On day 43, both groups of pigs were moved to an indoor finisher facility with a partially slatted concrete floor. Pigs were housed according to treatment, seven pigs to a pen. The finisher diet was a standard, commercial corn-soybean ration with no antibacterial agents. On day 140, pigs were sold to slaughter. No vaccinations were given to any of the offsite pigs during the study.

Onsite pigs

On day 61 of the study, 15 10- to 11-week-old pigs from the same farrowing cohort as the offsite pigs were moved from an inside nursery, ear tagged for identification, and moved to an outdoor grow-finish pen, where they were commingled with ≥ 100 other grow-finish pigs. These 15 pigs served as onsite controls.

Assessing *Salmonella* status

Offsite pigs

On the day after purchase (day 1 of the study), and on days 43, 83, and 109 of the study, each offsite pig was bled, and the sera frozen for later concurrent analysis for *S. choleraesuis* antibodies by ELISA, as previously described.[9] Also on days 1, 43, 83, and 109 of the study, two rectal and two oropharyngeal swabs were obtained from each pig for *Salmonella* culture.

Onsite pigs

Sera were taken from all 15 onsite control pigs on the day they were moved to the outside grow-finish pen (day 61), and from 13 of the 15 pigs on day 99. Serological samples were analyzed by ELISA. Two rectal swab samples were also taken from each of the 15 onsite control pigs on day 61 and from 13 of the onsite pigs on day 99.

Sows

On day 0 of the trial, two rectal swabs were collected from each of 24 sows housed in the farrowing unit from which the offsite pigs had been purchased. In addition, the udders of nine of the 24 sows were washed with soap and water, disinfected with 90% ethanol, and milk samples were collected in sterile containers for *Salmonella* culture.

Farm environment

On days 61 (n = 8), 99 (n = 6), and 217 (after marketing the offsite pigs) (n = 10), the following environmental samples were obtained from the farm of origin for *Salmonella* culture:

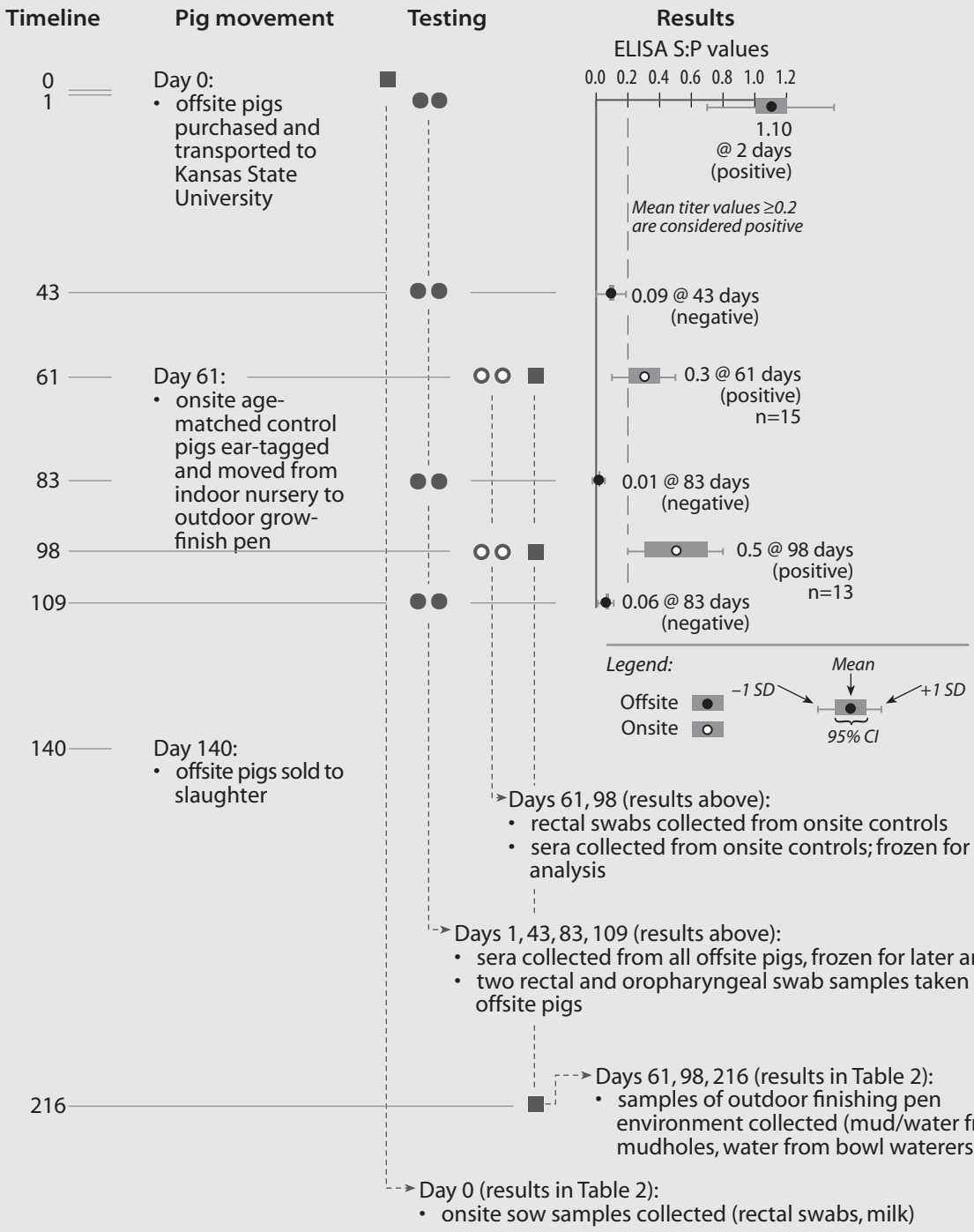
- water from bowl waterers;
- water/water-mud mixture from mud holes in the outdoor finisher pens.

Salmonella culture

One of each of the two swabs taken from onsite pigs (rectal), offsite pigs (rectal and oropharyngeal), and sows (rectal) was used to inoculate tetrathionate broth (Difco Laboratories, Detroit, Michigan), and the other swab was used to inoculate 3MC broth.[10,11] Similarly, approximately 1 mL of milk, water, and water-mud mixture was inoculated into 9 mL of the tetrathionate broth, and 1 mL into 9 mL of the 3MC broth. Each of these paired swab cultures were considered to be one sample.

The broths were incubated for 24 hours at 37°C and used to inoculate MacConkey agar (Difco) and Hektoen enteric agar (Difco) plates,

Figure 1



Study timeline and ELISA test results

which were incubated overnight at 37°C. Both tetrathionate and 3MC broths were held at room temperature for 5 days, after which 1 mL of each was used to inoculate new tubes of broth which were incubated overnight at 37°C.[12,13] These tubes were used to inoculate MacConkey and Hektoen agar plates, which were incubated overnight at 37°C. Lactose-negative clear colonies (MacConkey agar) and H_2S-positive green-black colonies (Hektoen enteric agar) were further characterized using standard microbiologic procedures. All *Salmonella* isolates were serotyped by the National Veterinary Service Laboratory, Ames, Iowa.

Serological analysis

All serological samples taken in this study were analyzed by ELISA test, as previously described.[9] Briefly, *S. choleraesuis*-soluble antigen was heat-extracted at 65°C for 1 hour from filtrates of cell supernatants.[9,14] This antigen contained LPS and multiple protein bands. The indirect

Table 2

Onfarm samples taken for culture and results

Date*	Sample	Source	Positives (n)	Number of isolates	Serotypes (serogroup)
June 28	rectal swabs	sows	1 (24)	1	*S. heidelberg* (B)
	milk	sows	0 (9)		
August 28	rectal swabs	age-matched onsite pigs	1 (15)	1	*S. agona* (B)
	mud/water	outdoor grow-finish environment	1 (8)	1	*S. derby* (B)
October 4	rectal swabs	age-matched onsite pigs	7 (13)	7	*S. agona* (B)
	mud/water	outdoor grow-finish environment	5 (6)	7†	*S. agona* (B) (n = 3) ; *S. derby* (B) (n = 2); *S. drypool* (E_2) (n = 2)
January 30	mud/water	outdoor grow-finish environment	5 (10)	6‡	*S. agona* (B) (n = 2); *S. choleraesuis* (C_1) (n = 1); *S. derby* (B) (n = 3)
Total			20 (85)	23	

† Two *Salmonella* spp. were isolated from each of the two samples
‡ Two *Salmonella* spp. were isolated from one sample

ELISA was performed using swine sera at a single 1:1000 dilution, horseradish peroxidase-labeled rabbit anti-pig IgG, developing reagents, and diluents according to the supplier's instructions (Pierce Laboratories, Rockford, Illinois). Antibody activity was expressed as sample:positive (S:P) ratio:

sample absorbance – negative control absorbence ÷ corrected positive absorbence

S:P values in excess of 0.2 were considered positive reactions.

Results

Offsite pigs

During the trial, two offsite pigs were euthanized, one because of persistent weight loss resulting from a gastric ulcer and one because of a rectal prolapse. Both pigs were necropsied and tissues cultured and examined microscopically. No evidence of salmonellosis was found. The remaining pigs remained healthy throughout the trial.

All 222 oropharyngeal and 222 rectal swabs taken from the offsite pigs were culture negative for *Salmonella* spp. On the day of purchase, ELISA S:P values of all but two of the pigs were in the positive range (> 0.2) with a mean of 1.1 ± 0.4 (SD) (Figure 1). By 43 days after purchase, the S:P value for all pigs had decreased and all were < 0.4 with a mean S:P value of 0.09 ± 0.09. At 83 days after purchase, all pigs were serologically negative and the mean S:P value had dropped to 0.01 ± 0.04. The mean S:P value had risen slightly to 0.06 ± 0.04 at 109 days after purchase, but all ELISA values remained < 0.2.

Onsite samples

Many of the onsite pig, sow, and environmental samples were culture positive for *Salmonella* spp. (Table 2). *Salmonella heidelberg* was isolated from the rectal swab of a single sow in the farrowing house, but was never isolated from the environment or on-farm nursery or finisher pigs. *Salmonella agona* was isolated from multiple mudholes and bowl waterers in the farm's outdoor finisher pens. One of the 15 age-matched pigs was culture positive for *S. agona* when moved from the nursery to an outdoor pen. Five weeks later, rectal swabs from seven of the 13 age-matched pigs were culture positive for *S. agona*.

On day 61, 13 of 15 onsite control pigs were serologically positive for *Salmonella* with a mean S:P value of 0.3 ± 0.2 (Figure 1). Five weeks later, the mean S:P value of the on-farm controls had risen to 0.5 ± 0.3, with S:P values > 0.2 for all but two pigs.

Between the purchase and sale of the offsite weaned pigs, *S. choleraesuis* was not isolated from the farm of origin, and the owners felt that clinical salmonellosis had ceased to be a problem. However, 2 and 3 months after the offsite weaned pigs had been marketed, *S. choleraesuis* was isolated from a bowl waterer in an outside finisher pen and from the large intestine of a finisher pig.

Discussion

These results demonstrate that it is possible to prevent *Salmonella* spp. infection of pigs originating from a heavily contaminated herd by raising them at a distant, clean site. We failed to isolate *Salmonella* spp. from any of the 444 rectal and oropharyngeal swabs from the offsite weaned pigs, but isolated one or more *Salmonella* spp. from seven of 15 (46.7%) age-matched, on-farm pigs and 12 of 57 (21.1%) on-farm environmental or sow samples. All 54 offsite weaned pigs that were serologically positive when purchased became serologically negative by 83 days after purchase and remained negative through 109 days after purchase.

Serologic testing by ELISA has been shown to be more sensitive than culture for identifying pigs and cattle that have been or are infected with *Salmonella* spp.[13,15,16] The antigen used in the ELISA test was prepared from *S. choleraesuis*, a serogroup-C_1 *Salmonella* spp. This antigen, however, has been found to cross-react with *S. typhimurium*, a serogroup-B *Salmonella* spp.[9] In the offsite weaned pigs, the presence of high titers at purchase followed by a uniform decline in titers 43 days later, and the uniformly negative titers at 83 and 109 days after purchase indicate that there was passive transfer of colostral antibodies from the sows to the newborn piglets, that over time the pigs lost these passively acquired antibodies, and that the pigs were not exposed to *S. choleraesuis* or to any of the four serogroup-B *Salmonella* spp. isolated from the on-farm pigs.

The ELISA titers of the onsite control pigs increased between days 61 and 99 of this study, indicating ongoing exposure to *Salmonella* spp. This exposure may have been the result of vaccination at weaning with the modified-live *S. choleraesuis* vaccine, SC-54, but we do not believe this to be true. Studies have shown that the titers of pigs vaccinated with SC-54 are not significantly different from the titers of nonvaccinated pigs when tested with the ELISA assay used in the present study,[17] or with the Danish MIX-ELISA for *Salmonella*.[18] The reason that pigs vaccinated with SC-54 do not develop anti-*Salmonella* antibodies is unknown, but it is possible that the vaccine stimulates the mucosal immune system rather than the humoral immune system.

Since the offsite pigs were all weaned on the same day, we were unable to evaluate the effect that age at weaning might have had on preventing infection. It is possible that if the pigs had been weaned and moved offsite at 3 weeks of age, which was the standard weaning age for the herd of origin, *Salmonella* infection would still have been averted. It is also possible that if multiple groups of pigs from the same herd had been weaned offsite over an extended period, not all groups would have remained *Salmonella* free. Other researchers have observed that *Salmonella* infection was not prevented in all groups of pigs weaned offsite.[7]

Carbadox was included in the diet of the offsite weaned pigs for the first 6 weeks because it is a standard component of the early weaning diet fed at KSU, and was added only for its growth promotion benefits.[6] Carbadox at the concentration used in this study has antibacterial activity against *Salmonella* spp. and is labeled for use in control of salmonellosis. Thus, a possible beneficial effect of carbadox cannot be totally discounted. However, we believe that removing the offsite pigs from the highly contaminated farm environment and raising them at a clean site was probably what successfully prevented them from becoming infected with *Salmonella,* rather than any effects of carbadox.

Because *Salmonella* infection was prevented in both the ceftiofur-medicated and nonmedicated offsite groups, we cannot conclude that ceftiofur played a significant role in preventing *Salmonella* infection in these pigs. Fedorka-Cray, et al.,[7] prevented *Salmonella* infection in several, but not all, groups of pigs without the use of antibacterial compounds, suggesting that medication may not be necessary.

Lactogenic immunity is thought to play an important role in protecting nursing pigs from clinical salmonellosis.[1,19] The presence or absence of shedding by the sows and the degree of contamination in the farrowing facilities may be more important in preventing transmission of *Salmonella* spp. to suckling piglets. Of the 24 rectal and nine milk samples from sows in the farrowing house, only one rectal swab was *Salmonella*-positive, and could only be isolated after 5 days of preenrichment, indicating that low numbers of organisms were present. This suggests that preventing vertical transmission and that cleaning facilities could play a vital role in any offsite finishing program. If the sows had been actively shedding large numbers of salmonellae, or if the farrowing house had been contaminated, it is unlikely that *Salmonella* infection could have been prevented in the offsite pigs. Fedorka-Cray, et al.,[7] observed that most of the pigs in their study that became infected with *Salmonella* were from sows that were culture positive.

Clinically normal carrier pigs that shed salmonellae in their feces are theorized to be important in transmission.[1] However, our findings indicate that once *Salmonella* spp. are introduced onto a farm, their presence in the environment can infect pigs, and thus identifying carrier pigs may not lead to elimination of the disease unless the facilities can also be adequately cleaned between groups of pigs. Thirteen *Salmonella* isolates were obtained from the 24 environmental samples taken from the on-farm finisher pens. When moved from the nursery to a finisher pen, only one of 15 (6.7%) onsite pigs was culture positive, but after 5 weeks in the outdoor pen, seven of 13 (53.8%) were culture positive. *Salmonella agona*, *S. derby*, and *S. drypool* were all isolated from the on-farm finisher pens. Even if there were no infected pigs coming from the nursery, the contaminated finisher pens could have provided a source of infection. Observations that both *S. choleraesuis* and *S. typhimurium* remained viable for at least 450 days after being buried in an Indiana sod pasture[20] suggest that contaminated dirt lots could remain a source of infection for a long period. The role of the farm environment in *Salmonella* transmission was also investigated in a recent study in which pigs raised in a barn with solid floors and open-flush gutters at the rear third of the pens were significantly more likely to be culture positive for *Salmonella* spp. than pigs in the same herd that were raised in a building with partially slotted floors, although there was not a significant difference in the *Salmonella* antibody titers of the two groups.[21]

Except for *S. choleraesuis*, none of the *Salmonella* spp. isolated from the on-farm samples is considered to be important swine pathogens.[1,2] However, all *Salmonella* spp. are regarded as potential human pathogens.[3] Reducing the numbers of *Salmonella*-infected pigs going to market is in the best interest of the swine industry. Management techniques and housing designs that reduce or eliminate fecal transmission between groups of pigs and pigs within a group have a beneficial effect on reducing *Salmonella* infection and are becoming increasingly important in preharvest food safety control programs.

Implications

- Offsite grow-finish offers pig producers a management tool that can be used in conjunction with other management techniques — such as all-in–all-out (AIAO) production and rigorous cleaning between

groups of pigs — to help control *Salmonella* infection.

- Moving pigs offsite at weaning may not always be effective, especially when the nursing sows are actively shedding salmonellae in their feces, or when the facilities cannot be cleaned sufficiently between groups. Also, salmonellae could be introduced through an outside source. However, if the pigs are moved AIAO and the facilities are rigorously cleaned between groups, the offsite grow-finish strategy could be reapplied.
- Preventing environmental cycling of *Salmonella* spp. will be an important component of preharvest food safety programs.

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