RESEARCH REPORT
PORK SAFETY

Title: Use of naturally-occurring bacteriophage to reduce *Salmonella* in swine prior to harvest.
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Abstract

Swine can be a reservoir of *Salmonella* that can be transmitted to human consumers of pork products. Bacteriophage are viruses that prey on bacteria and may be a potential strategy to reduce foodborne pathogenic bacteria in the gastrointestinal tract of food animals. Phages are fairly common in the gastrointestinal microbial ecosystem of mammals, but the incidence is unknown. If phages are to be an intervention strategy, we must understand their role in the microbial ecology of the gut. Therefore the current study was designed to determine the incidence of phage active against *Salmonella* spp. in the feces of commercial finishing swine in the United States. Fecal samples (n=60) were collected from each of six commercial swine finishing operations. Samples were collected from 10 randomly selected pens throughout each operation. Total number of fecal samples collected in this study was n=360. *Salmonella* spp. were found in 6.6% of the fecal samples. *Salmonella* spp. were isolated from only 2 farms and the serotypes represented were Schwarzengrund, Anatum, Ohio and Heidelberg. Bacteriophages were isolated from fecal sample through 2 parallel methods, 1) initial enrichment in *Salmonella* Typhimurium, or 2) initial enrichment in *E. coli* B (a strain very sensitive to phages); followed by direct spot-testing against *Salmonella* Typhimurium. Bacteriophages active against *Salmonella* Typhimurium were isolated from 1.1% (4/360) of the individual fecal samples when initially enriched in *Salmonella* Typhimurium, but *E. coli B*-killing phages were isolated from 43.8% (158/360) of the fecal samples but only 2 of these isolates were capable of killing *Salmonella* Typhimurium. Our results indicate that bacteriophage capable of killing *Salmonella* Typhimurium are fairly widespread across commercial swine production facilities but may be present at relatively low populations. When these pigs artificially infected with *Salmonella* Typhimurium were dosed with these phage isolates (10^9 PFU/pig) at 24 and 48 h prior to sacrifice, cecal populations of *Salmonella* were reduced slightly over 10-fold, and the number of pigs positive for *Salmonella* in the cecum were lower. *Salmonella* populations of the rectum were not changed by phage treatment, nor were the numbers of pigs containing *Salmonella* in the ileocecal lymph nodes. Phage isolates were used as an area spray to kill *Salmonella* on surfaces similar to lairage pens; however because these phage were isolated anaerobically, their efficacy under aerobic conditions was minimal. These results indicate that phage (predator) populations may vary along with *Salmonella* (prey) populations and that phage could potentially be used as a food safety pathogen reduction strategy.

III. Introduction

Food-borne *Salmonella* infections in the United States are estimated to cost the economy $2.4 billion annually (ERS/USDA, 2001). Approximately 6-9% of human salmonellosis is associated with the consumption of pork products (Frenzen et al., 1999). *Salmonella* is relatively common on swine farms and has been isolated from all stages of the pork production chain (Davies et al., 1999; Fedorka-Cray et al., 1997; Rostagno et al., 2003). *Salmonella* is a threat to the pork industry not only from a food-safety perspective as a public health concern, but some *Salmonella* serotypes can cause clinical illnesses in swine, negatively impacting production efficiency and profitability (Schwartz, 1991).

Bacteriophage are viruses that specifically infect bacteria and reproduce within them, killing the host bacterium through cellular lysis caused by the release of daughter phages. Phage were widely used in eastern Europe in place of antibiotics and have been called an "infectious cure for infectious disease" (Barrow, 2001). Due to increasing concerns about antibiotic resistance linkage to animal agriculture, considerable research has been focused on finding alternatives to antibiotics to reduce pathogens in food animals. Because phage exhibit a high degree of specificity for target bacteria it has been suggested that bacteriophage be used as a "designer antimicrobial" to eliminate specific pathogens from the gastrointestinal microbial population, including *Salmonella*, *Campylobacter*, *Listeria* and *E. coli* O157:H7 (Alisky et al., 1998; Loc Carrillo et al., 2005; Smith and Huggins, 1983).

Bacteriophage have been long known to be members of the intestinal microbial consortium. But the role they play in the ecology of the gut has been unclear; theories have abounded about preventing overgrowth to a role in diurnal variation to a role in nutrient cycling. However the exact incidence of these bacterial predators has never been fully investigated. Most research involving phages in the intestinal tract of animals has been exclusively qualitative in nature; incidence rates were determined using less than 20 animals of various species (Dhillon et al., 1976). Therefore this study was conducted to determine the incidence of phage in swine, by examining two separate issues, 1) what is the incidence of phage that kills Salmonella Typhimurium (one of the most important human illness serotypes in the United States), and 2) what is the overall incidence of bacteriophage in commercial swine.

IV. Objectives:

The objectives of this project were to: 1) isolate and characterize anti-*Salmonella* bacteriophage in commercial swine operations, 2) determine if these phage could be used in swine to reduce *Salmonella* populations in the intestinal tract, and 3) determine if phage could be used to decontaminate lairage pens at slaughter plants.

V. Materials and Methods: Objective 1

Fresh fecal samples (approximately 100 g from a single source; n = 6 samples per pen) were collected from each of 10 finishing pens per commercial swine farm (n = 10 pens/farm; n = 60 fecal samples/farm). Total number of fecal samples collected in this study was n=360.All samples were collected within a 45 min period immediately after the morning feeding. Immediately upon collection, samples were individually bagged in sealed whirl-pak bags after collection and kept on ice during transport prior to analysis (for approximately 24 h).

To qualitatively enrich for *Salmonella* populations, 3 g of feces were added to tubes containing 27 mL of tetrathionate broth (Difco Laboratories) and incubated at 37 ºC for 24 h. After this incubation, 200 µL of the tetrathionate enrichment were added to 5 mL Rapport-Vassilidis R10 broth and incubated an additional 24 h at 42 ºC before being streak-plated onto brilliant green agar (BGA) supplemented with novobiocin (25 µg/mL). The BGA_{Nov} plates were incubated for 24 h at 37 ºC; colonies that exhibited typical *Salmonella* morphology were individually picked for further physiological characterization and were inoculated onto Triple Sugar Iron (TSI) agar slants and Lysine Iron agar (LIA) slants (Difco, Inc.). Each slant was incubated at 35 ºC for 24 h. *Salmonella*-positive samples were confirmed by slide agglutination using SM-O antiserum poly A-I and V-I, and group C1 factors. *Salmonella* isolates were stored in glycerol and TSB at -80 ºC until confirmatory serotyping was performed by the National Veterinary Services Laboratory (NVSL) in Ames, IA.

<u>*Bacteriophage enrichment and isolation*</u>, Fecal samples were screened for the presence of *Salmonella* Typhimurium bacteriophage. Feces (1 g) were mixed in sterile conical tubes containing 9 ml of phosphate buffered saline (pH 6.8). Chloroform (0.5 ml) was added to each tube and tubes were thoroughly mixed before being allowed to stand at 24 °C for 2 h. The top layer from this tube was removed and placed in a new sterile tube containing 0.5 ml chloroform. Portions (0.3 ml) of the chloroform-free top layer were mixed with 1.2 ml volumes of early-log-phase (< 0.2 OD) *S.* Typhimurium or *E. coli* B (10^8 CFU/ml, grown at 39 °C) and were incubated in anoxic TSB broth in sealed Hungate tubes overnight at 39 °C. *E. coli* B was used in this study as an initial propogation strain because, 1) it is susceptible to bacteriophage of several types, and 2) use of this strain to propagate natural bacteriophage allows us to detect a broader range of phage in an initial bacteriophage activity screening. Samples (1.5 ml) were collected and added to tubes containing 0.2 ml of chloroform for 30 min. These samples were subsequently centrifuged at 19,000 x *g* in a

microcentrifuge for 10 min. The top layer of the supernatant was removed, and stored in a fresh sterile tube following sterilization by filtration through a 0.2 mm filter. Samples were subjected to a plaque assay (Sambrook and Russell, 2001) using *S.* Typhimurium or *E. coli* strain B as the propagation host and grown on TSB plates incubated anaerobically. Plates were incubated overnight at 39 °C.

Spectrum of bacteriophage activity, All bacteriophage plaques purified from the *S.* Typhimurium and *E. coli* B plates (3 plaques/sample) were assessed for their ability to form plaques on a range of intestinal bacteria. *E. coli* F18 and K88 were obtained from the FFSRU culture collection. Other bacterial species tested for bacteriophage activity included *Salmonella derby, S. typhimurium, S. dublin, S. enteriditis, S. cholerasuis, S. montevideo, S. mbandaka, Enterococcus faecalis, Entero. faecium* and *E. coli* O157:H7 from the FFSRU culture collection. Each bacterial strain was grown on TSB plates incubated anaerobically and were exposed to an equal amount of bacteriophage plaque forming units (PFU) of each bacteriophage isolate. The bacteriophage that were isolated in this study are currently being genetically and physiologically characterized and further characteristics of the bacteriophage will be reported in future studies.

Data Analysis, Point prevalence of *Salmonella* and bacteriophage shedding was calculated individually by dividing the number of pathogen culture-positive fecal samples by the total of samples collected per farm (n = 60 per farm, 360 total samples). Correlations of prevalence were using Epi Info 6.0, but due to the relatively low numbers of pens and incidences in this study, no correlations were found. Length of time spent in pen or farm were not included in the models because the record-keeping was not complete or available to the researchers.

Objective 2: Materials and Methods Bacteriophage that lysed *Salmonella* Typhimurium strains were collected from commercial swine (as described above). Phage isolates used in this study were selected for their activity against a *S.* Typhimurium commonly used in our laboratory in *in vivo* studies; isolates that created the largest plaques (clearing zones) on bacterial lawns were selected. The most active phages (4 isolates) were propagated individually in *S.* Typhimurium using a standard liquid amplification protocol. Briefly, phage stocks were prepared by adding plaques (MOI = 0.1-0.001) to a culture *S.* Typhimurium culture that was in early exponential growth phase (OD_{600} < 0.3). After growth was completed (overnight) chloroform was added to cultures followed by vigorous shaking to lyse bacterial cells, releasing phage into the media. Chloroform-treated samples were centrifuged at low speed (5000 x g, 10 min) to remove bacterial debris. The supernatant fluid contained free bacteriophage which could be used for assays or further amplification in subsequent cultures of *S.* Typhimurium.

All procedures in this study were approved by the Institutional Animal Care and Use Committee (IACUC protocol 05-001). Fourty six (n = 46) Yorkshire/Landrace cross pigs (average 15 kg initial BW) were purchased and transported to the FFSRU laboratory. Pigs continued to be fed a commercial swine grower ration formulated according to NRC recommendations and pigs were allowed water access *ad libitum*. Pigs were housed in environmentally controlled facilities and were screened for the presence of *S.* Typhimurium capable of growth on antibiotic supplemented agar and for the presence of bacteriophage that could lyse *S.* Typhimurium prior to experimental infection. Fourty-six (n = 46) pigs that contained neither phage nor antibiotic-resistant *S.* Typhimurium were randomly assigned to either treatment group (control or phage-treated).

S. Typhimurium cultures and inoculation *S.* Typhimurium was repeatedly grown by 10% (vol/vol) transfer in anoxic (85% N_2, 10% CO_2, 5% H_2 atmosphere) Tryptic Soy Broth (TSB) medium at 37 °C. This strain was made resistant to novobiocin (20 □ g/mL) by repeated transfer and selection in the presence of sub-lethal concentrations of each antibiotic. This resistant phenotype was stable through multiple unselected transfers in batch culture and through repeated culture vessel turnovers in continuous culture (data not shown). Overnight cultures (1000 mL) were harvested by centrifugation (7,500 x *g*, 10 min) and cell pellets were re-suspended in TSB medium (150 mL total volume). Each pig was inoculated with *S.* Typhimurium (3 x 10^{10} CFU) via oral gavage (10 mL total volume per pig) at -48 h. Fecal samples were collected via rectal grab 12 h after inoculation and subsequently at 12 h intervals and populations of inoculated *S.* Typhimurium were enumerated via 10-fold serial dilution and direct plating as described below.

Phage treatment. Individual phage isolate cultures were grown overnight in 1000 ml cultures of *S.* Typhimurium as described above. Chloroform was added to the cultures to release phage, and cultures were centrifuged to remove cellular debris. Phage supernatants were serially diluted and spot tested against lawns of *S.* Typhimurium to determine phage populations in the supernatant. Phages (n = 4 isolates at concentrations of approximately 10^8 PFU/ml) were pooled. Swine were dosed with phage cocktail via oral gavage (20 ml volume, 10^9 PFU/pig) at time 0 and 24 h. The estimated final phage concentration of approximately 10^4 PFU/ml intestinal contents per dosing.

Gastrointestinal sample collection. Swine were humanely euthanized and exsanguinated at 48 h (96 h after *S.* Typhimurium inoculation). Intestinal contents and epithelial tissues from the cecum and rectum, and ileocecal lymph nodes were aseptically collected upon necropsy. Samples were diluted as described below for quantitative enumeration of intestinal *S.* Typhimurium populations. Sample aliquots and epithelial tissues were added to tetrathionate for overnight

qualitative enrichment for inoculated *S.* Typhimurium. Overnight enrichments were plated as described below. Gastrointestinal content pH's were determined immediately upon return to the laboratory using a Corning 430 pH meter equipped with a calomel pH meter (Acton, MA). Intestinal contents were analyzed for volatile fatty acid (VFA) concentrations as previously described (Corrier et al., 1990)

Bacterial enumeration. Cecal, and rectal contents and feces were serially diluted (ten-fold increments) in phosphate buffered saline (PBS; pH 6.8). Dilutions were plated on Brilliant Green agar (BGA) supplemented with novobiocin (20 μg/ml) and incubated overnight at 37°C. Colonies that grew on agar plates after 24-h incubation were directly counted (quantitative enumeration). To qualitatively confirm the presence of inoculated *S.* Typhimurium, intestinal contents and epithelial tissue samples as well as feces were incubated overnight in Tetrathionate broth at 37°C, and were transferred subsequently to Rappaport-Vassiladis broth and incubated for 24 h at 42°C and were streaked on novobiocin supplemented BGA plates. Plates that contained colonies after 24-h incubation were classified as positive for inoculated *S.* Typhimurium.

Bacteriophage enumeration. Phage populations in intestinal contents of pigs were estimated by treating a (2 ml) aliquot of each dilution tube (above) with chloroform to lyse bacterial cells. The layer without chloroform was spotted (10 ml) on a bacterial lawn of *S.* Typhimurium. The presence of phage in a sample was determined by the presence or absence of plaques (clearing zones) in the lawn.

Objective 3: Materials and Methods. *Salmonella* Typhimurium were artificially inoculated in a slurry (25% added water vol/vol to aid in mixing and even spreading) of pig feces to reach a final concentration of 10^4 cfu/g fecal slurry. Phage were sprayed on at various concentrations and spread at a concentration of 10 g/100 cm^2 onto posterboard to simulate pen flooring during these preliminary studies. The phage concentrations were varied to obtained bacteria:phage ratios (or multiplicity of infections) of 1:1, 1:10, 1:100, and 1:1000 cfu:pfu. The phage spray was be allowed to remain on the fecal surface for 24 h. Samples were collected from each board, 3 samples per pen board (36 samples/treatment) using the methods described above.

Fecal samples were then spread onto concrete pen flooring to replicate studies replicating the above posterboard preliminary studies. Initial studies showed that concrete reduces inoculated S. Typhimurium populations about one $\log_{10}$/100 cm^2. Concentrations of S. Typhimurium and phage were increased to overcome this apparent bactericidal effect. The study above was repeated on the pen flooring.

To counteract the negative results in the first floor trials, the fecal slurry water level was increased to 40% and 50% water. This was in effort to 1) provide enough water for the phage to actively spread from infected host to host 2) neutralize any pH or chemical reaction.

Pigs (n=6) pigs were artificially inoculated with our *S.* Typhimurium strain and their feces was collected. The feces was diluted to obtain final concentrations of S. Typhimurium of $10^2 - 10^3$ cfu/g (approximately 40% added fluid). Procedures described above were repeated using this source of "naturally" inoculated fecal slurry.

VI. Results Objective 1:

Salmonella Enterica serotypes were found in 6.6% of the fecal samples (24/360). *Salmonella* spp. were isolated from only 3 of the 6 farms and the serotypes represented were Schwarzengrund, Anatum, Derby, Ohio and Heidelberg (Table 1).

Table 1. *Salmonella enterica* serotype, serogroup, phage active against *Salmonella* Typhimurium, and phage active against *E. coli* strain B isolated from commercial finishing swine in the central United States.

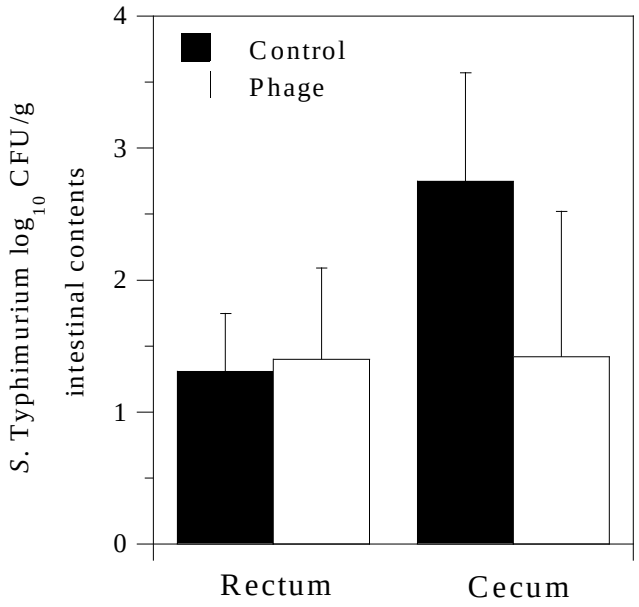
Farm	Serotype (number)	Serogroup	Phage + on *S.* Typh	Phage + on *E. coli* B
A	Anatum (1) Derby (1)	E1 B	0	0
B	None		3	18
C	None		0	13
D	None		1	7
E	Ohio (3) Heidelberg (1)	C1 B	2 (after B enriched)	60
F	Schwarzengrund (14) Anatum (4)	B E1	0	60
Total	**24/360**		**6/360**	**158/360**

Bacteriophages were isolated from each fecal sample through 2 parallel methods, 1) initial enrichment in *Salmonella* Typhimurium, or 2) initial enrichment in *E. coli* B (a strain very sensitive to phages) followed by direct spot-

testing against *Salmonella* Typhimurium. Bacteriophages active against *Salmonella* Typhimurium were isolated from 1.6% (6/360) of the total individual fecal samples, but *E. coli B*-killing phages were isolated from 43.8% (158/360) of the fecal samples. Only 2 of the *Salmonella*-killing phage were isolated from samples that were first enriched in *E. coli* B. All of these phages created clearing zones when plated onto *S.* Typhimurium and were characterized by their pattern against other *Salmonella* serotypes (data not shown). However, the spectrum of Salmonella-killing activity was very narrow, with only one of the phage killing another *Salmonella* serotype than Typhimurium (Derby).

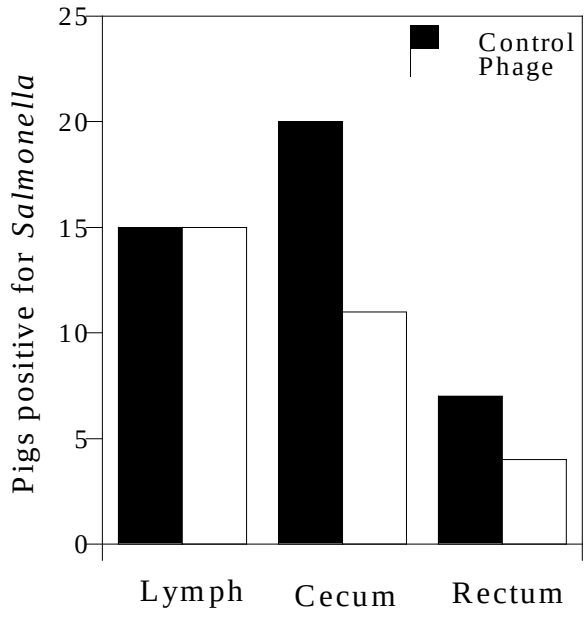
Results Objective 2: Phage treatment reduced concentrations of S. Typhimurium in used in pigs approximately 1.5 $\log_{10}$ CFU/g in cecal contents ($P < 0.1$).

Figure 1. Concentrations of *S*. Typhimurium in intestinal contents from n=23 pigs per treatment group.



The number of pigs that were positive for S. Typhimurium on direct plating and/or enrichment were lower in the cecum and rectum in phage-treated pigs, and neared statistical significance ($P < 0.1$) in the cecum.

Figure 2. Numbers of pigs positive for *S*. Typhimurium in ileocecal lymph nodes, cecal and rectal contents. In each treatment group total n = 23 pigs.



Results Objective 3: On posterboard: at this relatively low level of fecal slurry contamination, the 1:100 ratio was most effective, reducing *Salmonella* Typhimurium populations from 10^4 to 10^2 cfu/100 cm^2 in a 6 h period following phage application.

The reduction in *S.* Typhimurium populations when used on the concrete floor was likely due to desiccation of cells of our strain on the surface in this relatively dry slurry. In these studies, phage treatment caused no difference in the *S.* Typhimurium populations in the fecal slurry. When fecal concentrations of *S.* Typhimurium were increased to account for the desiccation from concrete, the phage again showed little/no effect. In follow-up studies, the phage ratios were again altered to find the optimal phage concentration. It was not possible to replicate the results from the posterboard on concrete. We hypothesize that the phage are being damaged by desiccation on the surface, surface pH or other chemical reaction with the concrete, or are simply unable to move through the 25% fluid slurry to reach target bacteria.

When pigs inoculated with *S.* Typhimurium feces was utilized, there was no effect of phage treatment on fecal populations of *S.* Typhimurium.

We were able to reduce phage from 10^3 to 10^2 cfu/100 cm^2 in a 50% water slurry. Unfortunately, this high of a level of water slurry is exceedingly unrealistic. Additionally, the level of phage that were used in this most successful trial 1:10,000 was accomplished by pouring phage solutions directly onto the flooring at 2 ml/cm^2. This is also exceedingly unrealistic and would not be feasible in the real world. Additionally, during these studies, we also reduced the time window to 6 from 24 h in case there was subsequent bacterial overgrowth (data not shown). No enhanced killing was found by narrowing this time window.

VII. Discussion

Salmonella spp. and other foodborne pathogenic bacteria can live in the gut of mammals, including swine. A wide variety of *Salmonella* serotypes have been isolated from swine around the world. The present study indicates that *Salmonella* are present in commercial finishing operations in the U.S. at a relatively low incidence that is comparable to other published surveys (Davies et al., 1999; Morrow et al., 1999). However, the fact that *Salmonella* are isolated from apparently healthy finishing swine has serious implications for pork safety; yet of the serotypes isolated in this study, only Anatum is found in the most common human isolates of the CDC. It is important to note that less than 7% of the fecal samples were positive for *Salmonella* spp., and these were limited to 3 of the 6 farms surveyed, indicating that herd health measures have indeed been effective in reducing the incidence of *Salmonella* in finishing swine.

Phages are normal members of the microbial ecosystem of the gastrointestinal tract of animals and humans, and are commonly isolated from community wastewater streams. In spite of understanding that phage are widespread in nature, no research has been performed to estimate the incidence of phage in food animals until recently; and never before

in commercial swine. The widespread nature of phage that were active against *E. coli* B was surprising, but the incidence varied between farms, from being ubiquitous on 2 farms to completely absent on one farm.

Phage that killed *Salmonella* Typhimurium were not as widespread on farms; only 6 out of the 360 samples tested positive for phage active against *S.* Typhimurium. Phage active against S. Typhimurium had a very narrow activity spectrum, and did not affect a variety of other *Salmonella* serotypes. Phage specific to *S.* Typhimurium were not widespread on these farms, likely because *S.* Typhimurium is not widespread on the farms for a *S.* Typhimurium phage to prey upon. These data suggest that in order to utilize phage to reduce *Salmonella* in swine, that a specific phage or phages be isolated for each specific serotype or group of related serotypes.

Phage have been suggested as a mechanism to reduce *Salmonella* spp. contamination in swine as an animal health adjunct, or as a potential preharvest intervention strategy. It appears that this strategy may be more difficult than previously considered due to the relatively narrow spectra of phage activity against the vast number of *Salmonella* serotypes (>2500 serotypes). In order to reduce *Salmonella* in the U.S. swine population, *Salmonella*-killing phage that affect the serotypes of interest must be isolated from several swine sources to reduce the possibility of resistance development and to ensure that the phage are effective against all strains of the serotypes of interest in the animal.

Bacteriophage show promise as use as an anti-*Salmonella* agent in swine. In this proof of concept study we found that cecal populations of *Salmonella* Typhimurium could be reduced by phage treatment. The number of animals that were colonized by *S.* Typhimurium were reduced by phage treatment in the cecum, and to a lesser extent in the rectum. Because so few phage isolates were found with activity against *Salmonella* spp. only a limited "cocktail" of phage were used in this study. As more anti-*Salmonella* phage are collected from swine in production settings, it is expected that more active phage will be found and these can be added to this phage cocktail to enhance its efficacy. Additional surveys to find phage will undoubtedly discover phage that are active against other *Salmonella* spp that impact pork production efficiency and port safety. Adding these phage isolates to an anti-*Salmonella* cocktail will widen the spectrum of activity, reducing the effects of *Salmonella* and the risks of developing phage-resistant *Salmonella* strains.

The use of phage as a pen cleaning agent failed in this study. However, in retrospect this is not surprising and should have been foreseen. The phage isolates in this study were selected primarily for use in the animal, in an anaerobic environment, the gut. The fact that the use of these anaerobic phage was unsuccessful was not a surprise given the aerobic environment of the pens. Additionally, phage move throughout their environment via Brownian motion (the motion of atoms in a liquid) and as such, there must be liquid to allow them to contact susceptible bacteria. The recent approval of phage sprays to eliminate *Listeria* and *E. coli* O157:H7 from various surfaces indicates that this approach has promise, but that specific selection criteria must be used to gather the appropriate isolates. This process is being currently repeated using highly aerated cultures of *Salmonella* to select phage types that are more effective in aerobic environments. But to date, no effective phage isolates have been found, but the work will remain in-progress as we search real-world isolates for further phage to utilize.

Conclusions

Our results indicate that bacteriophage are fairly widespread across commercial swine production facilities, but they may be present at relatively low populations. Phage capable of killing *Salmonella* Typhimurium are found in commercial swine, but were not found at a high incidence. This is potentially due to a predator/prey cycle between the phage (predator) and *Salmonella* (prey) populations. These results suggest that because this cycle naturally exists in the commercial environment, that phage could potentially be used as a food safety pathogen reduction strategy. In vivo studies indicated that phage could effectively reduce populations of *Salmonella* in swine intestinal tracts, as well as the number of pigs containing *Salmonella* Typhimurium. This study provides a proof-of-concept of phage utilization, but does not demonstrate a perfect efficacy from phage treatment. Further research is needed to understand the spectrum of activity of each phage type, and to specifically isolate phages active against the *Salmonella* spp. that most directly affect swine production efficiency, animal morbidity/mortality, and human food-borne illness/pork safety.

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VIII. Lay Interpretation

Salmonella is one of the most common causes of foodborne illness in humans and can also impact swine production efficiency. Bacteriophage are viruses found in nature that can kill bacteria, including *Salmonella*. Generic phages were found to be fairly widespread in commercial swine, but only 6 out of 360 commercial swine were positive for anti-*Salmonella* bacteriophage. When these phage were used in pigs that were artificially infected with Salmonella, the *Salmonella* populations in phage-treated pigs were lower compared to untreated controls. Additionally, fewer pigs were colonized by *Salmonella* in the cecum in the phage-treated groups. While we do not suggest that we have a complete solution for *Salmonella*, our data indicates that the concept of using phage to reduce *Salmonella* in swine is valid and feasible. We are continuing to examine commercial swine to obtain more potent anti-*Salmonella* phage to be able to maximize this potential pathogen reduction strategy. We gratefully thank the National Pork Board and our U.S. pork producers for their generous financial assistance in exploring this important area of improving food safety.

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Submitted Publications:

Initial data to be presented at the 2007 SAFEPORK Meeting in Verona, Italy.