

Fecal shedding of *Salmonella* by pigs housed in buildings with open-flush gutters

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Summary

Objective: To evaluate the possible role of recycled effluent in the epidemiology of fecal shedding of *Salmonella* by finishing pigs housed in barns with open flush gutters.

Methods: In six herds where finishing pigs were housed on solid concrete floors with open gutters (flushed with recycled lagoon water), locations of pigs with fecal samples positive for *Salmonella* were examined with respect to position in the barn and direction of effluent flow.

Results: In two herds (herds A and B), which received pigs and feed from the same sources, prevalence of positive fecal samples varied widely (60% and 4% respectively). In herd A (60% positive), gutters were flushed intermittently and the prevalence of pigs found to be shedding *Salmonella* was higher in downstream pens with respect to the flow of effluent. In herd B (4% positive), prevalence of fecal shedding was low, although gutters were flushed continuously with effluent recycled from single stage lagoon. In the other four herds, prevalence varied widely (4% to 59%) and no association between fecal shedding and pen location with respect to effluent flow was evident. In two herds, differences in serotype distribution between sides of barns suggested pen-to-pen transmission to be important. Clustering of positive pigs in some pens was also apparent.

Implications: The increased risk of *Salmonella* shedding by pigs in barns with open gutters observed in earlier studies may not be primarily attributable to the use of recycled lagoon water for flushing. Inefficient removal of fecal matter, resulting in increased transmission of *Salmonella* within and between pens, may be more important.

Keywords: swine, *Salmonella*, fecal shedding, food safety

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Outbreaks of human illness linked to consumption of animal products contaminated with bacteria, including *Salmonella enteritidis* in eggs,¹ verotoxigenic *Escherichia coli* O157:H7 in ground beef,² and *Listeria monocytogenes* in soft cheeses,³ have prompted discussion of the adequacy of conventional organoleptic

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methods of food inspection and suggestions that reducing foodborne pathogens in animal production systems is necessary to ensure safe food.⁴

In July 1996, the regulation and inspection of meat hygiene was radically altered in the United States by the passage of the Hazard Analysis Critical Control Point (HACCP)/Pathogen Reduction Act. One provision of this act was to establish targets for pathogen reduction based on standardized sampling protocols, defining limits for the allowable prevalence of positive cultures (*Salmonella*) or bacterial counts ('generic' *E. coli*, meaning any *E. coli* regardless of serotype). From January 1998, major slaughter plants must conduct daily microbial testing for generic *E. coli* as an index of fecal contamination of carcasses and thus of the adequacy of process control in the plant. Monitoring for *Salmonella* on carcasses is conducted by the Food Safety Inspection Service (FSIS) of the United States Department of Agriculture (USDA) to determine whether *Salmonella* prevalence on carcasses is below the target or if remedial measures are necessary for the slaughter process. Although this legislation does not directly address on-farm control of pathogens, one can expect that producers will be affected indirectly if slaughter plants that exceed FSIS targets for *Salmonella* prevalence begin to screen sources of pigs to avoid purchasing from herds in which prevalence is high.

In a recent review of foodborne diseases in the United States that were linked to red meat and poultry products,⁵ salmonellosis was ranked as the most important disease based on estimated costs of acute and chronic disease. This review also concluded that there is insufficient epidemiologic knowledge of many foodborne agents in animal production systems to enable reliable and cost-effective control measures to be implemented on farms. Most *Salmonella* infections of pigs are asymptomatic, with the possible exception of *S. choleraesuis* and *S. typhimurium*.⁶ Veterinarians and producers require better information about risk factors for asymptomatic *Salmonella* infection to enable them to implement appropriate control and prevention strategies.

Modern methods of raising pigs in multiple-site production systems using all-in–all-out (AIAO) management of finishing buildings, which have been effective in controlling some infectious diseases of growing pigs,⁷ appeared to have no benefit in reducing the prevalence of *Salmonella* compared with conventional farrow-to-finish systems.⁸ Housing finishing pigs on solid concrete floors with open-flush gutters increased the risk of *Salmonella* shedding compared with housing on slotted concrete floors.^{8,9} This study investigates the spatial locations, with respect to the flow of effluent in open flush gutter barns, of finishing pigs shedding *Salmonella* in feces.

Materials and methods

Herds

This study of six finishing herds is based on retrospective analysis of data from a previous study.⁸ Herds eligible for the current study were specialized finishing herds where barns with solid concrete floors and open-flush gutters were managed in an AIAO manner. In these herds, sample origin (feces from individual pigs) had been recorded by pen number so that locations of pigs shedding *Salmonella* in feces (hereafter termed 'positive pigs') could be identified. In each herd, the reference population for estimating *Salmonella* prevalence in the original study⁸ comprised all pigs (300–1000) in the barn housing the oldest pigs within 1 month of slaughter (as identified by the producer). The target sample size was 90 individual fecal samples, which enabled us to estimate the prevalence of positive pigs in the barn to within 10% at the 95% confidence level, assuming an expected prevalence of 50%.¹⁰ The number of samples collected per pen was determined by dividing the target sample size by the number of pens containing pigs. Some minor adjustments were made subjectively when the number of pigs per pen varied widely.

Facilities

Herd A

Herds A and B were associated with the same company, and received pigs and feed from the same sources. In each herd, the open gutters were flushed with lagoon water recycled from one single-stage anaerobic lagoon adjacent to the buildings. In herd A, the gutters were flushed intermittently every 2–3 hours. Barns in herd A had a central aisle separating two rows of 16 pens (Figure 1), with each pen containing approximately 20 pigs. Recycled lagoon water flowed through the building in open gutters at the peripheral end of the pens on each side of the building. Fecal samples were collected from three pigs in 26 of the pens and two pigs in six pens.

Herd B

Herd B received pigs and feed from the same sources as herd A. In contrast to herd A, lagoon water was pumped continuously through the gutters in the facility. The producer elected to do this because he believed that the increased cost of pumping was offset by the increased longevity of the pumps which were spared the repeated periods of high loading that occur with intermittent pumping. Herd B had 30 pens on the same side of the building (Figure 1), each containing approximately 20 pigs. Recycled lagoon water entered both ends of the building continuously and flowed to a drain in a pen in the center of the building. The depth of fluid in the gutter was estimated to be 5–8 cm (2–3 inches) on the day of sampling. In 26 of the 30 pens, fecal samples were collected from three pigs in each pen. The central pen with the open drain was not sampled to avoid risk of injury to pigs. To compensate, four pigs were sampled in three pens to achieve the target sample size of 90 pigs for the barn.

Herds C, D, E

In these herds, buildings had two rows of pens with a central aisle, and flush gutters running from one end to the other at the back of the pens (Figure 1). Herd C had 30 pens (approximately 20 pigs per pen); herd D had 26 pens (approximately 40 pigs per pen); and herd E had 36 pens (approximately 20 pigs per pen, with two pens empty). Herds C and D had multiple-stage lagoons, and herd E had a single-stage lagoon.

Herd F

Herd F had approximately 300 pigs housed in one row of 12 pens (10 smaller pens of approximately 20 pigs and two larger pens with 40 pigs) (Figure 1). Six pigs were sampled in the 10 smaller pens and 12 pigs in the two larger pens. Herd F had a single-stage lagoon.

Samples

Freshly voided, individual fecal samples were collected into Whirl-pak bags and transported to the laboratory to be processed the same day. Fecal samples (25 g) were cultured on XIT4 (Difco) and modified brilliant green (Oxoid) agars after pre-enrichment in buffered peptone water (Difco) and enrichment in Rappaport-Vassiliadis broth (Difco) as described previously.⁸ Colonies (one to three per plate) whose morphology was typical of *Salmonella* were transferred to triple-sugar-iron and urea agar slopes. Isolates (one per sample) identified biochemically as *Salmonella* were forwarded to the National Veterinary Services Laboratories in Ames, Iowa for serotyping. Associations between the proportion of positive samples and pen location in herd A were analyzed by χ^2 .

Results

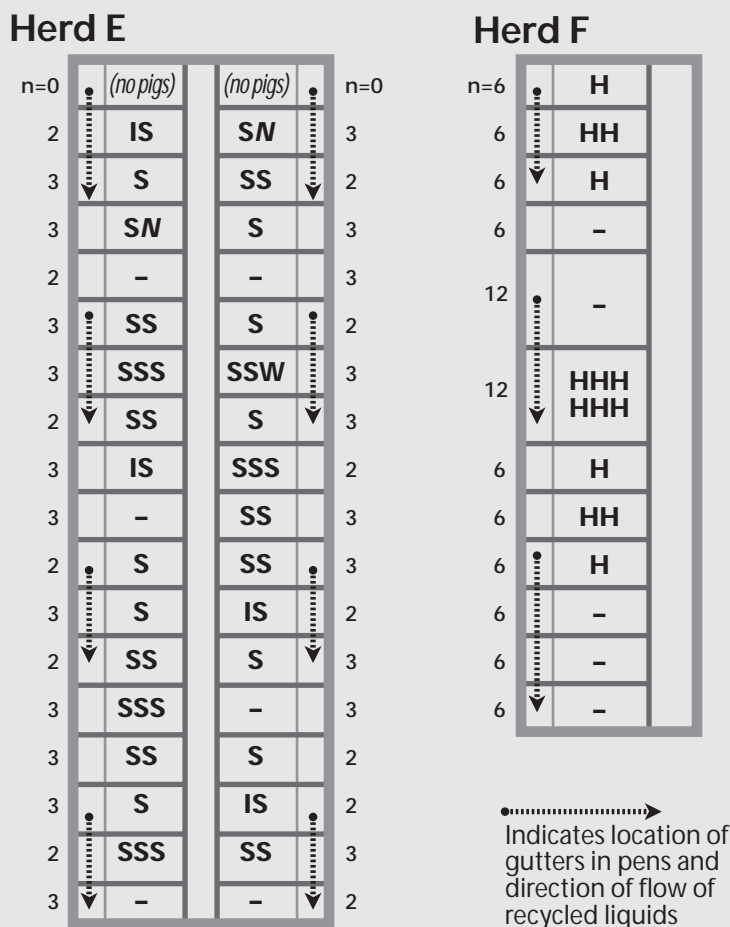
In herd A, 54 of 90 (60%) fecal samples were positive compared with four of 90 (4.4%) samples in herd B. *S. heidelberg* (n = 29) and *S. derby* (n = 23) were found in herd A (one sample contained multiple serotypes and another was contaminated) (Figure 1). At least one sample was positive in 26 of the 32 pens. The six pens from which all samples were negative were at the upstream end of the building where the flow of recycled lagoon water entered the building. The proportion of positive samples was dependent on their location in the barn ($P < .001$), and increased from the upstream to downstream ends of the building as follows:

- five of 23 samples (22%) from the eight (four per side) most upstream pens,
- 12 of 22 samples (55%) from the next eight pens,
- 16 of 23 samples (70%) from the following eight pens, and
- 21 of 22 samples (95%) from the eight most downstream pens.

The proportion of positive samples did not differ between sides of the building; however, the proportion of isolates of the respective serotypes was different ($P < .001$). Seven of 29 isolates (24%) from one side of the building were identified as *S. heidelberg*, compared with 22 of 25 (88%) isolates from the other side.

We observed no spatial patterns (with respect to effluent flow) of fecal

Figure 1, continued



Key to positive results

(each letter indicates one positive culture)

- | | |
|-----------------------------------|--------------------------------|
| A <i>S. agona</i> | T <i>S. typhimurium</i> |
| C <i>S. choleraesuis</i> | W <i>S. worthington</i> |
| D <i>S. derby</i> | |
| H <i>S. heidelberg</i> | M Multiple serotypes |
| I <i>S. infantis</i> | N Not serotyped |
| S <i>S. schwarzengrund</i> | X Contaminated culture |

shedding in herds B through F. All isolates from herd B were *S. typhimurium* and were collected from pigs in different pens (Figure 1). In herd C, which had a four-stage lagoon system and recycled water from the fourth stage, four of 90 samples (4.4%) were positive (all *S. choleraesuis*), again each from a different pen (Figure 1). In herd D, 52 of 88 (59%) samples (38 *S. schwarzengrund*, nine *S. typhimurium*, two *S. agona*, two *S. choleraesuis*, one multiple serotype), and 24 of 26 pens were positive (Figure 1). However, similar to the pattern seen in herd A, there was a difference ($P < .01$) in herd D between sides of the barn with respect to the proportion of isolates that were *S. schwarzengrund* (27 of 30 isolates on one side, compared with 11 of 22 isolates on the other side). Also, both isolates of *S. choleraesuis* were from the same pen, and six of the nine isolates of *S. typhimurium* were found in three pens (two per pen) indicating likely transmission within pens. For herd E, 52 of 89 samples (58%) were positive (45 *S. schwarzengrund*, four *S. infantis*, one

S. worthington, two not serotyped) and at least one positive sample was obtained from 28 of 34 pens. In herd F, 14 of 84 samples were positive (*S. heidelberg*) with no evident spatial distribution in the barn apart from an apparent clustering of positive samples in one of two large central pens (six of 12 samples positive, compared with eight of 72 pigs in eleven other pens).

Discussion

The importance of housing, particularly manure management and hygiene, in controlling *Salmonella* in swine (and other species reared under intensive conditions) has been recognized for many years.¹¹ The oral-fecal route is generally accepted to be the predominant route of transmission, although there is evidence of aerosol transmission.^{12,13} Management factors related to manure disposal and hygiene should, therefore, have a substantial impact on the risk of infection.^{11,14}

The present study used retrospective data available from an earlier study, which was not designed to define factors involved in *Salmonella* transmission among pigs housed in barns with open flush gutters. Recognized limitations of the data include

- the small number of herds, which were not selected randomly,
- inadequate sample size to estimate prevalence at pen level (as sample size was determined to estimate prevalence at the barn level), and
- sampling that was cross-sectional.

The inferences we've drawn from the data are therefore conditional, and this study should be regarded as a hypothesis-generating study directed at providing a foundation for more definitive studies.

In the United States in 1995, approximately 56% of finishing pigs were housed in total confinement and another 11% in open buildings with no outside access.¹⁵ Of all pigs finished, approximately 64% were raised on partially or fully slotted floors (predominantly concrete) and 31% were raised on solid concrete floors. Over 20% of all finishing herds, and 76% of herds marketing over 10,000 pigs per year, used anaerobic lagoons for manure storage.¹⁵ Recycled lagoon water is commonly used to flush effluent from beneath slotted floors or from open-flush gutters. Compared with using fresh water for flushing, flushing with recycled effluent reduces total effluent output from a herd, but clearly provides a potential vehicle for transmitting organisms shed in feces or urine.

Although *Salmonella* can survive in liquid and solid pig manure for several months, *Salmonella* concentrations in fresh effluent were found to be higher than in waste stored in anaerobic pits.¹⁶ After the separation of effluent into solid and liquid fractions, counts of *Salmonella* tend to be lower in the liquid than in the solid fraction.^{14,17} The decimal reduction time (time to reduce bacterial counts by 90%) is commonly used as a measure of bacterial survival in anaerobic digestion systems.¹⁷ These authors estimated that the decimal reduction time in a continuously fed full-scale digester, analogous to anaerobic lagoons common in North Carolina, was 34 days for *S. typhimurium*, considerably longer than earlier estimates made under experimental conditions.¹⁸ Factors thought to be important determinants of the survival of *Salmonella* in waste handling systems include:

- the percentage of solids and physical characteristics of slurry;
- source and rate of replenishment;
- storage temperature;
- initial concentration of organisms;
- pH, volatile fatty acids, and copper;
- competition with other organisms for nutrients; and
- aeration and possibly serotypes.^{14,16,17}

Given the multitude of factors that can affect *Salmonella* survival in anaerobic systems, we should expect risk of *Salmonella* infection of pigs that are exposed to recycled lagoon water to vary widely in commercial herds.

In a previous study of one herd, we found a higher prevalence of fecal samples positive for *Salmonella* among pigs kept in a continuous-flow barn with open-flush gutters compared to pigs kept on partially slotted floors.⁹ Similarly, across herds, prevalence was lower among pigs housed on slotted floors compared with all other floor types, including solid floors with open-flush gutters.⁸ Although it appears obvious that housing animals on solid floors with open gutters flushed with recycled effluent would favor transmission of organisms shed in feces, the actual mechanisms of transmission involved, and their relative importance, are not well defined. Potential contributing factors include:

- direct exposure to recycled, contaminated lagoon water;
- increased within-pen transmission owing to persistence of feces on solid floors; and
- increased pen to pen transmission via the common gutter.⁹

Our data suggest that the use of recycled lagoon water for flushing may be a relatively insignificant factor contributing to the increased risk of *Salmonella* infection in barns with open gutters. Firstly, low prevalence (4.4%) was found in two herds where pigs were exposed to recycled lagoon water, including herd B where exposure was continual and it was observed that pigs intermittently waded in and drank from the stream of recycled liquid in the open gutter. Secondly, the pronounced gradient we observed in the prevalence of positive pigs from the upstream to the downstream pens in herd A suggests that within- or between-pen transmission may have played an important role in this barn. Also, in two herds the predominance of different serotypes on each side of barns, despite exposure to common sources of lagoon water, suggests pen-to-pen transmission via open gutters, rather than

recycling of contaminated lagoon water, made an important contribution to the high prevalence of fecal shedding detected in those herds. Clearly, pigs in downstream pens were exposed to recycled water plus the effluent from all upstream pens, and thus were at greater risk of repeated exposure and reinfection. Also, one may hypothesize that pigs in downstream pens incurred greater risk of within-pen transmission because of the attenuation of the current of recycled water as it progressed along the length of the building. It has been suggested that all penmates of a pig shedding *Salmonella* may become infected,⁶ and our observation of clustering of positive samples within pens supports the importance of such transmission.

Hygiene at barn or pen level was not evaluated in this study, but inappropriate dunging behavior (dunging on solid sections of floors) in these types of facilities would be expected to markedly influence the risk of transmission. The use of recycled lagoon water has been implicated in the persistence of nonhost-adapted serotypes of *Salmonella* in dairy cows.¹⁹ Similarly, *S. agona* was isolated from recycled lagoon water entering a barn where this serotype had been found at a high prevalence, indicating the potential role for recycled liquids as a source of infection.⁹ The importance of recycled effluent as a source of infection, however, may be distinct from its role as a risk factor for high prevalence. In studies of dairy herds, the numbers of bacteria remaining on flushed surfaces did not vary significantly when fresh or recycled water was used for flushing.²⁰ Recycled liquids may introduce infection into an uninfected cohort of pigs (i.e., be a source), but other factors may determine the ultimate prevalence in the population, particularly if the risk of infection from recycled effluent is low relative to the risk of infection via other means (within-pen, pen-to-pen). The use of recycled lagoon water rather than fresh water to flush open gutters or pits has the advantage of reducing total effluent production and may play only a minor role in the epidemiology of *Salmonella*.

Implications

- Achieving frequent and effective removal of feces from the flush gutters by increasing the frequency or volume of flushing with recycled liquids may be as effective in controlling *Salmonella* transmission as substituting recycled effluent with fresh water for flushing.
- Facilities should be designed to enable effective removal of fecal material from pens to control *Salmonella* in barns with open-flush gutters. When constructing facilities with open-flush gutters, carefully design gutter volume, gradient, and flush volume to ensure effective removal of feces from all pens.

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