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Preslaughter Holding Environment in Pork Plants Is Highly Contaminated with *Salmonella enterica*

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The objective of this study was to determine whether abattoir pens can provide a *Salmonella enterica* infection source during the 2 to 4 h of preharvest holding. Previous work has suggested that pigs may be getting infected, but little has been reported on the environmental contamination of abattoir holding pens. For 24 groups of pigs studied (~150 animals/group) at two high-capacity abattoirs, six pooled fecal samples (*n*, 10 per pool) were collected from each transport trailer immediately after pigs were unloaded. Holding pens were sampled (one drinking water sample and six pooled floor samples consisting of swabs, residual liquid, and feces) prior to entry of study pigs for the routine holding period (~2.5 h). After slaughter, cecal contents and ileocecal lymph nodes were collected, on the processing line, from 30 pigs in each studied group. All samples were cultured for the isolation and identification of *S. enterica* by primary enrichment in GN-Hajna and tetrathionate broths, secondary enrichment in Rappaport-Vassiliadis broth, and plating on brilliant green sulfa and xylose-lysine-tergitol-4 agars, followed by biochemical and serological identification. The study pens were highly contaminated with *S. enterica*; all holding pens sampled had at least one positive sample. Additionally, 33% (8 of 24) of drinking water samples were positive for *S. enterica*. All 24 groups of pigs had *S. enterica*-positive cecal contents and ileocecal lymph nodes, including those groups from transport trailers with no positive samples. From pigs, trailers, and pens, 586 isolates representing 36 different *Salmonella* serovars were isolated. Of the 353 isolates from pigs (109 from ileocecal lymph nodes plus 244 from cecal contents), 19% were identified as belonging to the same serovars as those isolated from the respective pens; 27% were identified as belonging to the same serovars as those isolated from the trailers. Sixteen percent of the unique serovars were isolated from both pigs and pens, suggesting that pens served as the infection source. This study demonstrates highly contaminated abattoir holding pens and watering sources. It also demonstrates that holding pens can serve as an infection source. This study identifies the abattoir holding pens as a significant hazard and a potential control point for *Salmonella* contamination in the preharvest pork production chain.

Several studies have reported significantly higher *Salmonella enterica* prevalence rates in pigs tested at the abattoir than in pigs tested on the farm (4, 6, 8, 11, 12). This higher prevalence has been attributed to a nonspecific effect of stress from handling and transport. Stress is thought to affect the bacterial ecology of the gastrointestinal tract and the immunity of the animal, resulting in increased *S. enterica* shedding (7, 10). However, Hurd et al. (4, 6) have reported increased serovar diversity of isolates obtained after slaughter compared to that of isolates from pen mates necropsied on the farm. This increase in the diversity suggests that pigs may be exposed to new *S. enterica* infection sources after leaving the farm.

The slaughterhouse holding pen environment may serve as this nonfarm infection source. In the United Kingdom, long-term lairage (18 to 72 h) has been reported to increase *S. enterica* recovery rates (11). Little work has been reported on the *Salmonella* load in environments of U.S. pork plants. The holding pen environment may also be an important infection source in a short time period. Recent studies have demonstrated that under experimental conditions, *S. enterica* serovar Typhimurium can infect market age pigs exposed to a contaminated environment in as little as 2 h. *S. enterica* serovar Typhimurium was isolated from the feces and ceca 30 to 60 min postexposure (3, 5). Currently, most abattoirs avoid holding pigs more than 6 to 8 h. However, a minimum 2-h holding is encouraged to improve meat quality. This time is thought to be the minimum needed for pigs to recover from transport (17). Therefore, the objective of this study was to determine whether abattoir pens were contaminated at such a level as to provide an *S. enterica* infection source during the 2 to 4 h of preharvest holding. In previous studies (4, 6), pigs necropsied on the farm were compared to pen mates transported and held before slaughter. In the study reported here, we tried to eliminate the possible effect of transport stress (as a confounder variable) by testing pigs after transport.

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MATERIALS AND METHODS

Study design and sampling. Four groups of pigs (~150 animals/group) at each of two abattoirs (16,000 pigs/16-h workday) were sampled with three repetitions

at each abattoir (total, 24 groups). For each group studied, the transport trailer was sampled immediately after the pigs were unloaded. Samples consisted of six pooled fecal and bedding samples (10 samples per pool) taken from multiple random sites in the trailer. Trailers were selected so that all 150 pigs in the load were derived from the same farm. During the sampling, the truck drivers were interviewed to obtain the following information: (i) the time at which pigs were loaded at the farm; (ii) the time they arrived at the abattoir; (iii) the time pigs were unloaded; (iv) preparation of the trailers before loading (washing or no washing); and (v) the location of the farm (nearest town). The designated holding pens were sampled (six pooled samples per pen consisting of floor swabs, residual liquids, and/or feces—samples were pooled two samples per pool) prior to the entry of the study pigs into the pens. All samples were collected by using sterile gloves, gauzes, and syringes, placed in sterile plastic bags, and vigorously shaken for homogenization. Additionally, a sample of the drinking water from each pen was collected directly from troughs by using a sterile syringe. After slaughter, cecal contents and ileocecal lymph nodes (ICLN) were aseptically collected, on the processing line, from 30 pigs randomly selected from each group. Individual sets of sterile forceps and scissors were used to avoid cross contamination.

Sample processing. All samples were processed by standard bacteriological methods for the isolation and identification of *S. enterica*, as previously described (6). Briefly, the culture methods applied included primary enrichment in tetrathionate (48 h at 37°C) and GN-Hajna (24 h at 37°C) broths, secondary enrichment in 10 ml of Rappaport-Vassiliadis broth (24 h at 37°C), and plating on brilliant green sulfa and xylose-lysine-tergitol-4 agars (24 h at 37°C). All bacteriological media, as well as the *S. enterica* antiserum used during the sample processing, were obtained from Becton Dickinson Microbiology Systems, Sparks, Md. All trailer, pen, and cecal content samples (10 g or ml) were directly inoculated into 100 ml of each preenrichment broth. ICLN were immersed in 70% ethanol, flamed, and macerated in sterile bags with a rubber mallet; peptone water (10 ml) was added, and each sample was homogenized by using a stomacher (260 rpm for 1 min). After homogenization, 1 ml of supernatant was directly inoculated into 9 ml of each preenrichment broth.

For the transport trailer and holding pen samples, one to five suspect colonies were selected per plate, and for the pig samples (ICLN and cecal contents), one to three suspect colonies were selected per plate for *S. enterica* identification (biochemical and serological). Suspect colonies were presumptively identified in triple sugar iron agar and lysine iron agar slants. Isolates presumptively identified as *S. enterica* were stored in Trypticase soy agar slants and submitted for serotyping to the USDA National Veterinary Service Laboratories in Ames, Iowa.

Statistical analysis. Variables analyzed included the proportions of positive samples by abattoir (A and B), by sample source (trailers, pens, or pigs), and by group. The data analysis included frequency distribution analysis for each variable, cross tabulations, and comparison of proportions by using chi-square test (significance level, $P < 0.05$). Each group of pigs and each serovar isolated per group represented an opportunity or trial to test the null hypothesis that pens could not serve as an infection source. Every time a pen-specific serovar was isolated from pigs, it was considered to be a rejection of the null hypothesis that pens could not serve as an infection source.

RESULTS

The frequency of *Salmonella*-positive transport trailers, holding pens, and groups of pigs, as well the number of positive samples from each source, is presented in Table 1. All of the 24 pens had at least one positive sample; 83% of the trailers had at least one positive sample. Fewer *S. enterica* isolates were from the trailers; 43.8% of the 72 samples tested were positive, compared to 77.8% of the 72 pen samples. Additionally, 8 (33.3%) of the 24 holding pens studied had positive drinking water samples. *S. enterica* serovars isolated from these water samples included Typhimurium, Derby, Heidelberg, and Anatum.

There were significantly fewer ($P < 0.05$) *S. enterica* isolates at abattoir A than at abattoir B from trailers (34.7% versus 52.8%) and pens (65.3% versus 90.3%) (Table 1). Conversely, there were fewer *S. enterica* isolates from ICLN collected at abattoir B (7.2%) than from those collected at abattoir A (19.4%; $P < 0.05$). The percentages of positive cecal contents were similar at both abattoirs (32.2% versus 28.9%).

TABLE 1. *S. enterica* isolation and prevalence in transport trailers, holding pens, and slaughtered pigs (cecal contents and ICLN) at two abattoirs

Source	No. positive for *S. enterica*/total (%) at abattoir(s):		
	A	B	A and B
Holding pens	12/12 (100.0)	12/12 (100.0)	24/24 (100.0)
Pen samples	47/72 (65.3)[a]	65/72 (90.3)	112/144 (77.8)
Transport trailers	9/12 (75.0)[a]	11/12 (91.7)	20/24 (83.3)
Trailer samples	25/72 (34.7)[a]	38/72 (52.8)	63/144 (43.8)
Drinking water	3/12 (25)	5/12 (41.7)	8/24 (33.3)
Groups of pigs	12/12 (100.0)	12/12 (100.0)	24/24 (100.0)
Cecal contents	116/360 (32.2)	104/360 (28.9)	220/720 (30.6)
ICLN	70/360 (19.4)[a]	26/360 (7.2)	96/720 (13.3)

[a] Values for abattoirs A and B are statistically different ($P < 0.05$) by chi-square.

Hygiene was variable among trailers and abattoirs. Of the 24 sampled transport trailers, only seven (29.2%) were reportedly washed before pigs were loaded at the farm. All 12 of the pens in abattoir A were washed with high-pressure cold water before study pigs entered, whereas the pens from abattoir B were never washed.

The study pigs reportedly spent an average of 2.4 h (range, 0.5 to 10.15 h) in the transport trailers, from loading at the farm to unloading at the abattoir. The average pen holding time was 3.5 h (range, 1.9 to 5.3 h). There was no correlation between time spent in pens and recovery of pen-specific serovars from the pigs.

From pigs, trailers, and pens, 586 isolates representing 36 different *S. enterica* serovars were isolated. The 12 serovars most frequently isolated were Derby (24.1%), Anatum (19.6%), Typhimurium (Copenhagen) (18.6%), Saint-Paul (10.1%), Infantis (5.1%), Heidelberg (4.9%), Senftenberg (4.1%), Bovismorbificans (2.7%), Agona (1.4%), Minnesota (1.4%), Uganda (1.2%), and Mbandaka (1.2%). We isolated serovar Choleraesuis (Kunzendorf) from ICLN of three different pigs, two from the same farm.

The serovars isolated from pigs were categorized as matching those from the pens, the trailers, or both the pens and the trailers (Table 2). In 18 of the 24 groups of pigs (75%), we isolated at least one serovar matching serovars isolated from the pens or from both the pens and the trailers. For five groups of pigs, serovars isolated matched only those isolated from the corresponding trailers. In only one group of pigs (A11), there were no serovars that matched those from the trailer or the pens.

In 37.5% of the groups (9 of 24), we isolated serovars from the ICLN that were found only in the respective pens and not in the respective trailers. Serovars most frequently isolated from the 109 positive ICLN were Derby (37.6%), Typhimurium (Copenhagen) (20.2%), Saint-Paul (18.3%), Anatum (10.1%), and Choleraesuis (Kunzendorf) (2.8%).

Of the 353 isolates from pigs (109 from ICLN plus 244 from cecal contents), 19% were identified as belonging to the same serovars as those isolated from the respective pens; 27% were identified as belonging to the same serovars as those isolated from the trailers (Table 2). Twenty-three percent of isolates from pigs matched serovars from both the pens and trailers.

TABLE 2. *S. enterica* serovars[a] isolated from transport trailers, holding pens, and pigs that had spent time in holding pens

Abattoir and group	Holding time (h:min)	Serovar(s) (no. of positive samples) isolated from:				Source(s) of sample(s) with serovar(s) matching that from pigs[e]	No. of serovars per group from any sample
		Trailer[b]	Pen[c]	Pig ICLN[d]	Pig cecal contents[d]		
A1	3:15	TYC (2)	MBA (1), ANA (1)	TYC (13), TYP (1)	TYC (11)	Trailer	4
A2	2:37		ANA (1), DER (2), MON (1)	AGN (1), DER(7), TYC (1)	AGN (1), DER (5), TYC (1)	Pen	5
A3	2:22	DER (1), TYC (1)	TYC (1)	DER (11), TYC (2)	DER (16), TYC (10)	Both	2
A4	2:30		DER (1), MNS (1), MON (1), OHI (2), TYC (1)	ANA (1), DER (1)	MBA (2), MON (1), OHI (1), TYC (1)	Pen	7
A5	4:04	ANA (3)	ANA (2), DER (4), INF (1), MIN (3), SPA (2)	DER (3), INF (1), TYC (3)	ANA (4), DER (5), TYC (5)	Both	6
A6	4:05	ANA (2)	ANA (2), BRA (1), DER (2), INF (1), SPA (2)	ANA (1), CHK (1), DER (1)	ANA (5), DER (1), HEI (1), TYC (2)	Both	8
A7	1:55	HEI (1), KRE (1)	AGN (1), ANA (4), MIN (1), RDG (1)	ANA (1), DER (1)	ANA (3), DER (7)	Pen	7
A8	1:55		ANA (6), DER (2), MIN (1)	ANA (2), DER (1)	ANA (2), DER (3), TYC (1)	Pen	4
A9	3:55	ANA (1), TYC (1)	INF (1), SEN (1), TYC (4)	TYC (2)	ANA (1), DER (1), MPH (1), TYC (4)	Both	6
A10	2:00	GIV (1), SPA (5)	JOH (1), LIT (2), TYC (1), UGA (1)	SPA (6)	INF (2), SPA (9)	Trailer	7
A11	2:15	AGN (2)	DER (2)	ANA (1), HAR (2), SPA (8), TYC (1)	HAR (1), SPA (13)	None	6
A12	2:30	AGN (1), INF (2), MBA (2), MNS (1), TYC (1), WOR (1)	SEN (1), TYC (3)	MOL (1), SPA (4), WOR (1)	BAB (1), INF (1), SPA (9)	Trailer	10
B1	4:18	ANA (1), RMP (1)	ANA (6), HEI (1)	ANA (2), DER (1)	ANA (16), BOV (1), DER (1), NEW (1)	Both	6
B2	3:49	DER (1), TYP (2)	ANA (5), TYC (1)	ANA (1), CHK (2), DER (2), NEW (1)	ANA (6), DER (4)	Both	7
B3	4:00	BOV (1), DER (1), HEI (3)	ANA (6), DER (1)	ANA (1), AUT (1), DER (11), HEI (1)	ANA (4), BOV (1), DER (10), HEI (1), NEW (1)	Both	6
B4	3:34	HEI (6), LIV (1), OHI (1)	ANA (4), DER (1), HEI (1)	HEI (1)	DER (1), HEI (11), TYC (2), TYP (1)	Both	7
B5	4:08	INF (3)	ANA (1), DER (1), MIN (2), MUE (1), TYC (3)		INF (1), TYC (5)	Both	6
B6	4:06	INF (4)	ANA (3), TYC (4)	ANA (1)	CER (1), INF (3), OHI (1), TYC (1)	Both	5
B7	3:40	INF (1)	ANA (2), MIN (1), TYC (5)	DER (2), HEI (1), SPA (1)	DER (1), INF (7), PMO (1)	Trailer	8
B8	3:20	MON (1), SEN (5)	ANA (2), DER (1), INF (1), TYC (5)		ANA (1), DER (2), TYC (1)	Pen	6
B9	5:20	FAL (1), SEN (2)	CER (1), DER (5), MBA (2), TYC (1)		SEN (4)	Trailer	6
B10	5:05		ANA (2), DER (3), INF (1), TYC (3)		BOV (2), TYC (2)	Pen	5
B11	4:15	KEN (1), SEN (6)	ANA (3), DER (5)	SPA (1)	AGN (1), ANA (1), BOV (7), DER (1), SEN (1), TYC (1)	Both	8
B12	4:20	DER (1), KEN (1)	ANA (2), DER (1), OHI (1), TYC (1), TYP (1), UGA (5)		BOV (1), DER (1), SEN (3), TYC (1)	Both	9
Total		73	160	109	244		151

[a] ANA, Anatum; AGN, Agona; AUT, 4, 12, autoagglutinable; BAB, Babelsberg; BRA, Brandenburg; BOV, Bovismorbificans; CER, Cerro; CHK, Choleraesuis (Kunzendorf); DER, Derby; FAL, Falkensee; GIV, Give; HEI, Heidelberg; INF, Infantis; JOH, Johannesburg; KEN, Kentucky; KRE, Krefeld; LIT, Litchfield; LIV, Livingstone; MBA, Mbandaka; MIN, Minnesota; MNS, Muenster; MON, Montevideo; MOL, Molade; MPH, 4, 12, 1-monophasic; MUE, Muenchen; NEW, Newport; OHI, Ohio; PMO, 6, 7, poorly motile; RDG, Reading; SEN, Senftenberg; SPA, Saint-Paul; TYC, Typhimurium (Copenhagen); TYP, Typhimurium; UGA, Uganda; WOR, Worthington. Samples were collected from two commercial abattoirs on four different visits to each abattoir.

[b] Six pooled fecal and bedding samples were obtained from each trailer immediately after study pigs were unloaded.

[c] Six pooled samples per pen, consisting of floor swabs, residual liquids, and feces, were obtained prior to the entry of the study pigs into the pen. Pen results also include results for drinking water samples.

[d] Samples were taken, during processing, from 30 of the approximately 150 pigs on each trailer.

[e] Both, trailer and pen.

The remaining 31% of isolates did not match serovars from pens or trailers.

Every serovar isolated, per group, could have been isolated from the trailer, the pens, the pigs, or any combination thereof. For example, serovars from pigs could match those from the pen, those from the trailer, both, or none. Overall, there were 151 opportunities or trials to test the null hypothesis that pens could not serve as an infection source (Table 2). Figure 1 shows how these serovars were distributed among the following seven possibilities: (i) serovars from pens and pigs matched (16%); (ii) serovars from trailers and pigs matched (9%); (iii) serovars from all three sources, pigs, pens, and trailers,

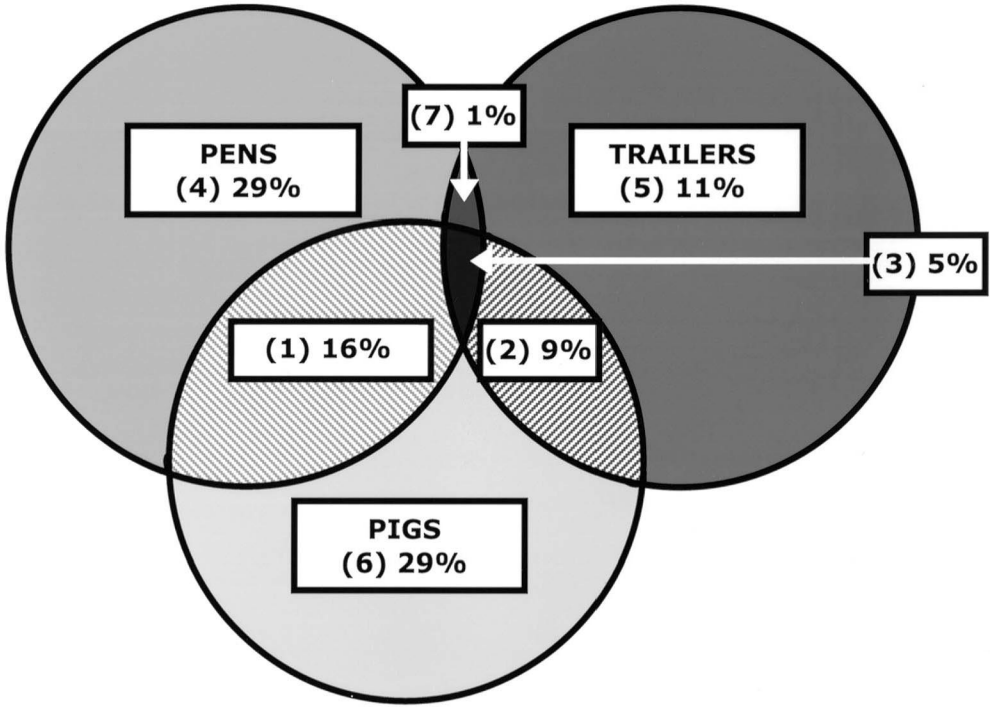

FIG. 1. Distribution of 151 unique *S. enterica* serovars isolated from transport trailers, holding pens before pigs entered, and pigs (ICLN and cecal contents) after holding. Categories are as follows: (1) serovars from pens and pigs matched (16%); (2) serovars from trailers and pigs matched (9%); (3) serovars from all three sources, pigs, pens, and trailers, matched (5%); (4) serovars from pens did not match any others (29%); (5) serovars from trailers did not match any others (11%); (6) serovars from pigs did not match those from pens or trailers (29%); and (7) serovars from pens and trailers matched one another but matched no serovars from pigs (1%).

matched (5%); (iv) serovars from pens did not match any others (29%); (v) serovars from trailers did not match any others (11%); (vi) serovars from pigs did not match those from pens or trailers (29%); and (vii) serovars from pens and trailers matched one another but matched no serovar from pigs (1%). Every time a serovar was isolated from the pigs and pens but not the trailers, there was the possibility that the serovar was derived from the pens, i.e., that the pens served as the infection source. In Fig. 1, it can be seen that 16% of the unique serovars were isolated from both pigs and pens, suggesting that pens served as the infection source.

DISCUSSION

The objective of this study was to evaluate whether preslaughter holding pens at pork plants could serve as an *S. enterica* infection source after the presumed stressful effect of transport had produced any additional shedding. It was not designed to quantify the difference in prevalences among transported and held pigs. The observation that pigs were infected with pen-specific serovars demonstrated that holding pens can provide an infection source immediately prior to slaughter. Additionally, this study demonstrates the high level of environmental *S. enterica* contamination.

The possible role of holding pens in increased prevalence of *Salmonella* infection in slaughtered pigs was first considered by Williams and Newell (18). However, these authors attributed greater importance to on-farm infection and transport stress. In our study, pigs spent an average of 2.4 h (with a minimum of 30 min) in the transport trailers and an average of 3.5 h in holding (minimum of 1 h, 55 min). These observations are critical, as Hurd et al. (3, 5) reported rapid infection in market weight pigs (as found in ICLN, feces, and cecal contents) 2 h after exposure to a contaminated environment. Feces and cecal contents were positive after only 30 to 60 min of exposure. Based on these observations, we conclude that the exposure time, in trailers or pens, was sufficient to allow infection or contamination of the gastrointestinal tracts.

There was a significant difference ($P < 0.05$) between abattoirs in the numbers of positive holding pen samples (62.5% in abattoir A and 90.3% in abattoir B), suggesting that washing reduced the *S. enterica* load. Abattoir A pens were washed with high-pressure cold water before study pigs entered, whereas abattoir B pens were never washed. In abattoir B, manure control was achieved by continuous sprinkler operation and well-drained pen floors. In both abattoirs, pens were continuously used and pigs from several different farms spent preslaughter holding time in these pens every day. This common practice in swine abattoirs allows a buildup of *Salmonella* populations in the holding pen environment, which constitutes a potential infection source for each new group of animals introduced into the contaminated pens. *Salmonella* has the ability to survive in many environmental niches, with great potential for persistence and dissemination (13). *Salmonella* has been demonstrated to survive for weeks, or even months, in the environment or in fecal material (1, 2). The proportion of positive trailer samples was higher in abattoir B, suggesting

that pigs arrived at abattoir B with higher prevalences. However, although abattoir B had more positive trailer samples, there was no difference between abattoirs in the prevalences of positive pigs, as measured by using cecal content samples, and the number of positive ICLN was actually lower at abattoir B. Additionally, it should be noted that pen samples were taken before the study pigs entered the tested pens, so correlations between pen and pig prevalences were not direct. More studies are needed to understand the environmental ecology of *S. enterica* and the effect of cleaning and disinfection.

In addition to the frequent contamination of the holding pens, the finding that 33.3% of the drinking water samples were contaminated with *Salmonella* was a surprise. This finding indicates that more attention to the microbiological quality of the water in abattoirs is needed or that the water may have been contaminated from the environment. The contamination of the troughs from the environment seems to be more likely, as almost all serovars found in the water samples were also isolated from the floor samples collected from the respective pens. Whatever the case, the drinking water available for the pigs during the preslaughter holding constitutes a potential (and very likely) infection source, justifying more attention.

No matches were observed for 29% of the *Salmonella* isolates collected from pig samples (Fig. 1, category 6). This observation may have been due to the lack of shedding or to sampling limitations. Different sample types were collected from the trailers (pooled feces and bedding), pens (floor swabs, residual liquids, and feces), and pigs (cecal contents and ICLN). Insufficient sampling of the transport trailers and holding pens may have allowed some serovars to remain undetected. It is not clear whether the culture methods used in this study were efficient in detecting all *Salmonella* serovars present in the studied samples. According to Waltman (16), the media and methods that are best with one particular serovar may not necessarily be optimal for others. The number of suspect colonies selected for serotyping from each sample could have affected the proportion of serovars found. However, this does not seem to be the case as, in total, up to 20 suspect colonies were selected for identification from trailer and pen samples and up to 12 suspect colonies were selected for identification from pig samples. Even so, these potential limitations did not preclude comparisons, as the same methods were used for all samples.

It is evident that *S. enterica* is widely distributed in the abattoir lairage environment. In addition to our findings, Swanenburg et al. (15) reported high rates of *Salmonella* contamination in holding pens from two abattoirs in Europe. Similar results were reported for the holding pens of cattle and sheep abattoirs in the United States (14).

It appears that pigs became infected during preslaughter holding through exposure to the highly contaminated environment. The 16% of unique serovars isolated from both pens and pigs indicate that the abattoir holding pens provided that contaminated environment. Determining unequivocally the origin of isolates from the pigs is beyond the methods employed in this study. Further refinement is needed. Numerous genotyping methods have been applied to the typing of *Salmonella*, allowing the comparison of isolates and the allocation of strains with identical typing patterns into the same group. Tracking specific strains and determining infection source(s) constitute some of the most common applications of the combination of conventional and molecular epidemiological data. However, at present, there is no consensus as to which molecular typing technique is best suited for intraserovar differentiation. For the time being, the most reliable and effective approach to fingerprinting of *Salmonella* for epidemiological investigations seems to be a combination of methods, which has to be assessed for every specific situation (9).

From the results presented and discussed here, it can be concluded that the preslaughter holding pen environment of pork plants is highly contaminated with *Salmonella*. Additionally, our results indicate that the contaminated environment of the holding pens can be a significant source of *S. enterica* infection for swine prior to slaughter. This study adds one more piece of information identifying abattoir holding pens as a significant hazard and potential control point for *S. enterica* contamination in the preharvest pork production chain.

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REFERENCES

1. **Baloda, S. B., L. Christensen, and S. Trajcevska.** 2001. Persistence of a *Salmonella enterica* serovar Typhimurium DT12 clone in a piggery and in agricultural soil amended with *Salmonella*-contaminated slurry. Appl. Environ. Microbiol. **67:**2859–2862.
2. **Gray, J. T., and P. J. Fedorka-Cray.** 2001. Survival and infectivity of *Salmonella* Choleraesuis in swine feces. J. Food Prot. **64:**945–949.
3. **Hurd, H. S., J. K. Gailey, J. D. McKean, and M. H. Rostagno.** 2001. Experimental rapid infection in market swine following exposure to a *Salmonella* contaminated environment. Berl. Muench. Tieraerztl. Wochenschr. **114:**382–384.
4. **Hurd, H. S., J. D. McKean, I. V. Wesley, and L. A. Karriker.** 2001. The effect of lairage on *Salmonella* isolation from market swine. J. Food Prot. **64**:939–944.
5. **Hurd, H. S., J. K. Gailey, J. D. McKean, and M. H. Rostagno.** 2001. Rapid infection in market-weight swine following exposure to a *Salmonella* Typhimurium-contaminated environment. Am. J. Vet. Res. **62**:1194–1197.
6. **Hurd, H. S., J. D. McKean, R. W. Griffith, I. V. Wesley, and M. H. Rostagno.** 2002. *Salmonella enterica* infections in market swine before and after transport and holding. Appl. Environ. Microbiol. **68**:2376–2381.
7. **Isaacson, R. E., L. D. Firkins, and R. M. Weigel.** 1999. Effect of transportation and feed withdrawal on shedding of *Salmonella* Typhimurium among experimentally infected pigs. Am. J. Vet. Res. **60:**1155–1158.
8. **Kim, J. Y., P. B. Bahnson, H. F. Troutt, R. E. Isaacson, R. M. Weigel, and G. Y. Miller.** 1999. *Salmonella* prevalence in market weight pigs before and after shipment to slaughter, p. 137–139. *In* P. B. Bahnson (ed.), Proceedings of the 3rd International Symposium on the Epidemiology and Control of *Salmonella* in Pork. Washington, D.C.
9. **Liebana, E.** 2002. Molecular tools for epidemiological investigations of *Salmonella enterica* subspecies *enterica* infections. Res. Vet. Sci. **72:**169–175.
10. **McGlone, J. J., J. L. Salak, E. A. Lumpkin, R. I. Nicholoson, M. Gibson, and R. Norman.** 1993. Shipping stress and social status effects on pig performance, plasma cortisol, natural killer cell activity, and leukocyte numbers. J. Anim. Sci. **71:**888–896.
11. **Morgan, I. R., F. L. Krautil, and J. A. Craven.** 1987. Effect of time in lairage on caecal and carcass *Salmonella* contamination of slaughter pigs. Epidemiol. Infect. **98:**323–330.
12. **Morrow, W. E. M., P. R. Davies, T. See, J. Eisemann, K. Zering, S. Kihlstrom, and K. Karli.** 1999. Prevalence of *Salmonella* spp. in the feces on farm and ceca at slaughter for a cohort of finishing pigs, p. 155–157. *In* P. B. Bahnson (ed.), Proceedings of the 3rd International Symposium on the Epidemiology and Control of *Salmonella* in Pork. Washington, D.C.
13. **Murray, C. J.** 2000. Environmental aspects of *Salmonella*, p. 265–283. *In* C. Wray and A. Wray (ed.), *Salmonella* in domestic animals. CABI Publishing, Wallingford, United Kingdom.

14. **Small, A., C.-A. Reid, S. M. Avery, N. Karabasil, C. Crowley, and S. Buncic.** 2002. Potential for the spread of *Escherichia coli O157*, *Salmonella*, and *Campylobacter* in the lairage environment at abattoirs. J. Food Prot. **65:**931–936.
15. **Swanenburg, M., H. A. P. Urlings, D. A. Keuzenkamp, and J. M. A. Snijders.** 2001. *Salmonella* in the lairage of pig slaughterhouses. J. Food Prot. **64:**12–16.
16. **Waltman, W. D.** 2000. Methods for cultural isolation of *Salmonella*, p. 355–405. *In* C. Wray and A. Wray (ed.), *Salmonella* in domestic animals. CABI Publishing, Wallingford, United Kingdom.
17. **Warriss, P. D., S. N. Brown, J. E. Edwards, M. H. Anil, and D. P. Fordham.** 1992. Time in lairage needed by pigs to recover from the stress of transport. Vet. Rec. **131:**194–196.
18. **Williams, L. P., and K. W. Newell.** 1968. Sources of salmonellas in market swine. J. Hyg. **66:**281–293.