PEER REVIEWED ORIGINAL RESEARCH

Genotypic and phenotypic comparison of swine *Salmonella* isolates from farm and abattoir

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Summary

Objectives: To determine if pigs became infected with different serotypes of *Salmonella* following transport and lairage, and whether genotypic and phenotypic analysis would identify different subtypes within the same serotypes.

Methods: *Salmonella* organisms were isolated from lymph nodes of pigs from three herds (A,B,C) at the abattoir, and isolates were compared to those previously cultured from fecal samples from two of the herds (A,B). The farms were located in three different states within the United States, and pigs were transported to the same abattoir in separate clean and disinfected vehicles. Serotyping, antibiotic resistance profiling, and genetic fingerprinting using pulsed-field gel electrophoresis were used to compare isolates.

Results: *Salmonella* serovars recovered from feces of pigs from farms A and B were also recovered from lymph nodes of pigs from the same farm. Additionally, isolates cultured from pigs at the abattoir were distinguishable from those cultured from pigs on the source farm by identification of serovars, genetic fingerprinting within serovars, and antibiotic resistance profiles.

Implications: Biosecurity, from the time market pigs leave the farm up to and including the hours immediately prior to slaughter, is crucial to the control of salmonellae in pork. New populations of salmonellae, not previously present on the source farm, may be recovered from pigs at the abattoir. Rapid infection may occur in pigs not previously harboring *Salmonella* organisms, and the strain may be multi-antibiotic resistant, further elevating the food safety risk.

Keywords: swine, *Salmonella*, food safety, pulsed-field gel electrophoresis, antibiotic resistance

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Understanding the epidemiology of human foodborne pathogen transmission through the "farm to fork" continuum is crucial when considering food safety in the livestock industry. In 1987, Morgan et al[1] described the effect of time in lairage on *Salmonella* contamination of slaughter pigs. They showed that the percentage of pigs in which salmonella organisms could be cultured from the cecum increased directly with the time spent in lairage. It was suggested that both the size of the holding pen and its hygiene contributed to this result. In turn, a high isolation rate from the cecum was generally associated with *Salmonella* recovery from the carcass.[1]

In 1995, Fedorka-Cray et al[2] described an alternate route of invasion in which *Salmonella* serovar Typhimurium was isolated from lymph nodes and cecum 3 hours after intranasal inoculation of esophagotomized pigs. Recently, *Salmonella* infection has been shown to occur in pigs less than 2 to 3 hours after they were placed in pens which previously housed *Salmonella* infected pigs.[3] In addition, a greater prevalence of *Salmonella* serovars, including serovars not recovered from cohort pigs at the farm of origin, have been reported in pigs at slaughter.[4] Recent work by others suggests that acute infection might play a role in contamination of pigs at the slaughterhouse.[5,6] Thus, it appears possible that, from the time they leave the farm until they are slaughtered, pigs from *Salmonella*-free herds may become contaminated through direct contact with contaminated trucks or facilities, or by commingling with *Salmonella*-positive pigs.[4,7]

In this three-herd study, we confirmed the previous work regarding new serovars being acquired in lairage. Our objective was to determine if new clones within serovars, distinguishable by genetic analysis and antibiotic profiling, were being acquired in lairage as well.

Materials and Methods

Sample collection

From July 1999 to July 2000, pooled pen fecal (PPF) samples were cultured approximately every other month from Herd A located in Kentucky and from Herd B located in Oklahoma. On each occasion, 20 PPF samples were collected, each consisting of 5 g of feces from five different places within a pen, for a total of 25 g. The sampled pens were chosen randomly and contained approximately 25 pigs weighing nearly 110 kg each. The samples were placed on ice and shipped overnight for

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culture within 24 hours of collection. In addition, during the course of this study, pigs that were sick or died were routinely necropsied, and tissues were submitted to a diagnostic laboratory for culture.

Approximately monthly from January 2000 to May 2000, 50 to 100 ileocecal lymph nodes were collected at an abattoir in Missouri from market weight pigs of Herds A and B, weighing approximately 120 kg and identified by tattoo. Pigs were transported from their respective farms to the same abattoir via separate clean and disinfected vehicles. Pigs used in the study were the first to be slaughtered that day and were held in lairage for at least 3 hours. Lymph nodes were also collected from pigs designated "Herd C," originating in Missouri and representing all other pigs killed that day. After Herd A, B, and C carcasses had been eviscerated, approximately 5 g of ileocecal lymph node was collected from each pig (n=359) and placed on dry ice to be cultured within 12 hours.

Table 1: *Salmonella* isolates cultured from pooled pen feces (PPF) collected from market-weight pigs on Farms A and B, and from ileocecal lymph nodes (ICLN) collected at the abattoir from cohort pigs

Sample source	No. of samples collected (No. positive)	No. of *Salmonella* isolates[1]	*Salmonella* serovar isolated (No. isolated)
Herd A			
PPF	161 (4)	21	Heidelberg (21)
ICLN	228 (8)	9	Heidelberg (9)
Herd B			
PPF	94 (40)	121	Heidelberg (82), Typhimurium (30), Worthington (9)
ICLN	90 (71)	69	Heidelberg (7), Typhimurium (16), Typhimurium var. copenhagen (1), Worthington (43), Derby (2)
Herd C[2]			
PPF	ND[3]	ND	ND
ICLN	41 (3)	9	Heidelberg (3), Infantis (3), Derby (3)

1 After pre-enrichment in buffered peptone water, samples were transferred to enrichment media (Rappaport-Vassiladis broth and xylose lysine deoxycholate agar), and positive samples were confirmed to be *Salmonella* serovars using biochemical tests and agglutination with specific antisera. If present, multiple *Salmonella* suspect colonies were chosen for further diagnostic testing.

2 Pigs at abattoir from sources other than Herds A and B.

3 ND: not determined.

Culture

Pooled pen fecal samples were diluted 1:10 in buffered peptone water (BPW; Difco, Detroit, Michigan). Lymph nodes were dipped in 70% ethanol for 2 seconds, flamed to decontaminate, macerated, and diluted 1:10 in BPW. Both fecal and lymph node BPW suspensions were incubated for 24 hours at 37°C, then 0.1-mL samples of the BPW suspensions were transferred for selective enrichment in Rappaport-Vassiladis (RV) broth (Difco), which was incubated at 42°C for 24 hours. A 0.1-mL sample of each BPW suspension was also plated on selective media, xylose lysine deoxycholate agar (XLD), at 37°C for 24 hours. A maximum of five *Salmonella* suspect colonies per XLD plate were inoculated into tubed media (Kliglers, sulfide indole motility, phenylalanine, and lysine iron tubes) and were tested for agglutination with *Salmonella* O antisera (Difco).[8] All *Salmonella* isolates were serotyped at the National Veterinary Service Laboratories, Ames, Iowa.

Antimicrobial sensitivity testing

Antibiograms were determined using the Kirby-Baur disk diffusion method.[9] *Salmonella* isolates were suspended in Mueller-Hinton (MH) broth (Difco), and turbidity was adjusted to 0.5 MacFarland Standard. Cultures were then inoculated onto MH plates and antimicrobial discs were applied (BBL, Cockeysville, Maryland). Plates were incubated at 37°C for 24 hours, and the zone of inhibition was interpreted according to the manufacturer's instruction. Isolates were checked for resistance to the following antibiotics: amoxicillin-clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, cefoxitin, ceftazidime, ceftiofur, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, colistin, enrofloxacin, furazolidone, gentamicin, kanamycin, naladixic acid, neomycin, piperacillin, piperacillin-tazobactam, spectinomycin, streptomycin, sulfisoxazole, tetracycline, ticarcillin-clavulanic acid, trimethoprim, and trimethoprim-sulfamethoxazole.

Genotypic analysis

Pulsed-field gel electrophoresis (PFGE) of *Salmonella* serovar Heidelberg isolates (n=68) was conducted using the endonuclease *Xba*I according to the protocol utilized by the Centers for Disease Control and Prevention.[10] The PFGE patterns were analyzed visually and also compared using BioNumerics software (Applied Maths, Kortrijk, Belgium). Dendrograms were constructed by the unweighted pair group method using arithmetic averages, Dice coefficient, and 0.8% optimization with 1.0% band position tolerance.[11]

Results

Salmonella serovars present in PPF samples from Herds A and B were also present within lymph nodes of pigs from the respective herds at slaughter (Table 1). Additional serovars and different subtypes within serovars were also isolated from lymph nodes of pigs from Herds A and B. Serovars that had not been isolated from PPF samples on the farm during the time frame of this study were isolated from pigs of Herd B (Table 1). In addition, serovars with antibiotic profiles not previously identified in the herd were found in lymph nodes at slaughter (Table 2). In both Herds A and B, at least one *Salmonella* subtype isolated from lymph nodes was resistant to more antibiotics than any subtype isolated from the respective herd PPF samples.

Salmonella Heidelberg was present in PPF samples of pigs from both Herds A and B and also from the lymph nodes of pigs from Herds A, B, and C, and was thus further analyzed by PFGE (Figure 1). A single PFGE pattern represented all of the *Salmonella* Heidelberg isolates from Herd A PPF samples. While this same pattern occurred in isolates from Herd A lymph nodes, isolates with a unique PFGE pattern were also present that were only 95% related. A

Table 2: Antibiotic resistance profiles[1] of select *Salmonella* serovars isolated ante mortem and post mortem from cohort swine

Pig Source	*Salmonella* serovar	Ante mortem[2]	Post mortem[3]
Herd A	Heidelberg	KST (5) KNeST	KST KNeST KSSuT* KCNeSSuSpT*
Herd B	Heidelberg	KST KNeST None[4]	KST KNeST
	Typhimurium	APiSSpSuT ANePiSSpSuT AKNePiSSpSuT ACeNePiSSpSuT	AKNePiSSpSuT AKSSpSuT* ACpKNePiSSpSuT*
Herd C	Heidelberg	ND[5]	KNeST

1 Ampicillin (A), cefepime (Ce), cephalothin (Cp), chloramphenicol (C), kanamycin (K), neomycin (Ne), piperacillin (Pi), spectinomycin (Sp), streptomycin (S), sulfisoxazole (Su), tetracycline (T).

2 Pooled pen feces were collected on the farm and cultured by pre-enrichment in buffered peptone water, selective enrichment in Rappaport-Vassiladis broth, and selective plating on xylose lysine deoxycholate (XLD) agar. *Salmonella* serovars were confirmed with biochemical tests and specific antisera agglutination.

3 Ileocecal lymph nodes were collected at the abattoir, dipped in 70% ethanol and flamed to decontaminate surface, macerated, and cultured as described for fecal samples.

4 Not resistant to any antimicrobial (Kirby-Baur disk diffusion).

5 Not determined.

* Not recovered from source herd.

single PFGE pattern also represented all *Salmonella* Heidelberg isolates from the Herd B PPF samples, although this pattern was slightly different from that of the Herd A PPF samples. This pattern was also observed in the isolate from the Herd B lymph nodes, but again a second pattern occurred. This new pattern was less than 80% related to the isolate from Herd B, but was exactly the same as the PFGE pattern of *Salmonella* Heidelberg isolates from lymph nodes of pigs from Herd C. No relationship was seen between serovar antibiotic profiles and PFGE patterns.

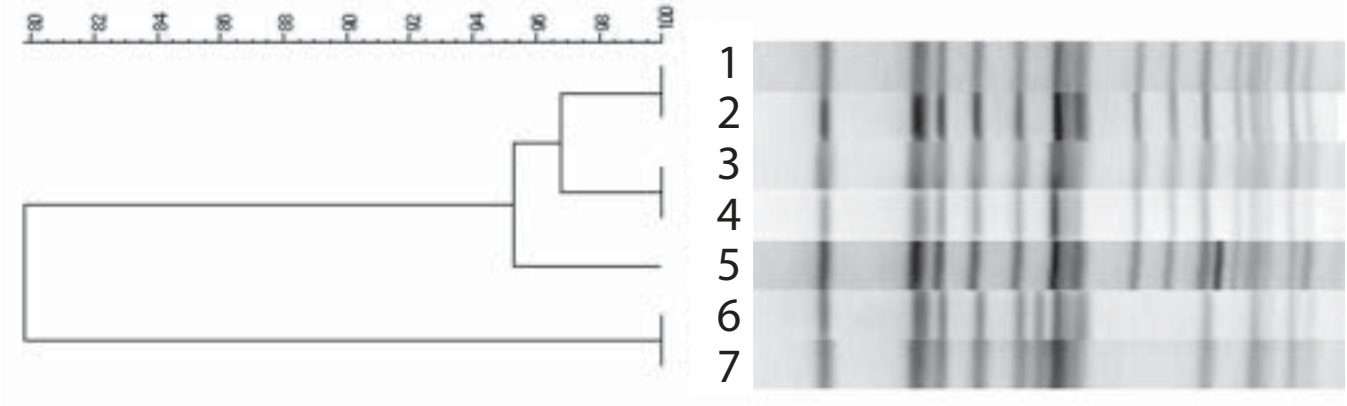
Salmonella was isolated from the lymph nodes submitted to a diagnostic laboratory from two Herd A pigs from that had been necropsied on the farm. The isolates were *Salmonella* Heidelberg and had PFGE patterns identical to the on-farm fecal isolates (data not shown).

Discussion

We found, in pigs at slaughter, *Salmonella* serovars and subtypes of serovars (distinguishable either by molecular genetic analysis or antibiotic resistance profiles) that were different from serovars found in the feces of cohort pigs at the farms of origin. The *Salmonella* Heidelberg isolates from farm and abattoir originating from the same source, while closely related, were still distinguishable by PFGE profiling. Previous work has shown a significant increase in *Salmonella* prevalence in lymph nodes of pigs slaughtered at an abattoir compared to those slaughtered on the farm from the same cohort.[4] The isolation of different *Salmonella* serovars from the same cohort of pigs at the abattoir supports previous work showing an increase in serodiversity following transport and lairage.[4] Between the time when the pigs left the farm and when they were slaughtered, they became infected with an additional *Salmonella* serovar, and likely new subtypes within serovars, that rapidly disseminated to the ileocecal lymph nodes.

This study assumed that the *Salmonella* serovars and genotypes detectable in feces are representative of the isolates present in lymph nodes in pigs at the herd of origin. This is a reasonable assumption based on the work of Wood et al,[12] who found that the strain of *Salmonella* Typhimurium used to inoculate 7- to 8-week-old pigs was

Figure 1: Pulsed-field gel electrophoresis (PFGE) patterns and resulting dendrogram of ante mortem and post mortem *Salmonella* Heidelberg isolates digested with restriction enzyme *XbaI*. Lane 1 represents pooled pen feces isolates from Herd A (n=18), and Lanes 2 and 5 represent lymph node isolates from Herd A (n=9). Lane 3 represents pooled pen feces isolates from Herd B (n=31), and Lanes 4 and 7 represent lymph node isolates from Herd B (n=7). Lane 6 represents lymph nodes isolates from Herd C (n=3).



consistently cultured from the feces as well as from the lymph nodes of pigs necropsied up to 28 weeks post-exposure. A recent study did indicate that no *Salmonella*, or fewer serovars, were present in feces compared to lymph nodes when pigs were necropsied on the farm.[4] However, in that study, only 1-gram samples of feces were collected from individual pigs, rather than pooled pen fecal samples. Funk et al[13] showed that a 25-gram sample of feces is far superior to a 1-gram sample for *Salmonella* detection. In addition, when pigs were necropsied on the farm and samples were subsequently submitted to a diagnostic laboratory for culture, *Salmonella* isolates from the lymph nodes were the same as those from PPF samples of pigs in the same cohort.

Efforts to reduce *Salmonella* rely on establishment of intervention strategies. The results of this study confirm the work of others,[2–4] ie, we conclude that acute *Salmonella* infection during transport and lairage requires intervention in order to control *Salmonella* contamination of pork. If indeed pigs exposed to *Salmonella* hours before slaughter can become systemically infected, pigs from *Salmonella*-free sources, or sources with a low prevalence of salmonellae,[14] may pose the same threat to food hygiene as pigs from highly contaminated systems. In addition, pork may become contaminated with organisms that are resistant to more antibiotics, thus posing a further threat to human health.

Implications

- *Salmonella* serovars and subtypes within serovars not previously identified on the farm of origin can be recovered from pigs at the abattoir.
- Pigs from herds with a low prevalence of *Salmonella* that come in contact with contaminated trucks, facilities, or pigs may pose the same risk to food safety as those from herds with a high prevalence of *Salmonella*.
- The time immediately prior to slaughter, including both transport and lairage, should be considered a key intervention point for control of *Salmonella* in pork production.

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