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

Matthew M. Erdman, BS; Stephanie D. Wedel, BS; D.L. Harris, DVM, PhD

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.

In 1995, Fedorka-Cray et al. 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. 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. Recent work by others suggests that acute infection might play a role in contamination of pigs at the slaughterhouse. 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.

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 genotypic and phenotypic comparison of swine *Salmonella* isolates from farm and abattoir. J Swine Health Prod. 2003;11(4):169-172.
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.

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, flame-decontaminated, 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 specific antisera. If present, multiple Salmonella suspect colonies were chosen for further diagnostic testing.

Antimicrobial sensitivity testing
Antibiograms were determined using the Kirby-Baur disk diffusion method. All 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, cefsulodin, ceftriaxone, cephalothin, chloramphenicol, ciprofloxacin, colistin, enrofloxacin, furaloxacin, furazolidone, gentamicin, kanamycin, nalidixic 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 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

<table>
<thead>
<tr>
<th>Sample source</th>
<th>No. of samples collected</th>
<th>No. of Salmonella isolates</th>
<th>Salmonella serovar isolated (No. isolated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPF</td>
<td>161 (4)</td>
<td>21</td>
<td>Heidelberg (21)</td>
</tr>
<tr>
<td>ICLN</td>
<td>228 (8)</td>
<td>9</td>
<td>Heidelberg (9)</td>
</tr>
<tr>
<td>Herd B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPF</td>
<td>94 (40)</td>
<td>121</td>
<td>Heidelberg (82), Typhimurium (30), Worthington (9)</td>
</tr>
<tr>
<td>ICLN</td>
<td>90 (71)</td>
<td>69</td>
<td>Heidelberg (7), Typhimurium (16), Typhimurium var. copenhagen (1), Worthington (43), Derby (2)</td>
</tr>
<tr>
<td>Herd C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPF</td>
<td>ND (3)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>ICLN</td>
<td>41 (3)</td>
<td>9</td>
<td>Heidelberg (3), Infantis (3), Derby (3)</td>
</tr>
</tbody>
</table>

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.

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 from 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
A 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.

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. 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. 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, who found that the strain of *Salmonella* Typhimurium used to inoculate 7- to 8-week-old pigs was

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

<table>
<thead>
<tr>
<th>Pig Source</th>
<th><em>Salmonella</em> serovar</th>
<th>Ante mortem</th>
<th>Post mortem</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herd A</td>
<td>Heidelberg</td>
<td>KST</td>
<td>KST</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KNeST</td>
<td>KNeST</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KSSuT*</td>
<td>KCNesSuSpT*</td>
</tr>
<tr>
<td>Herd B</td>
<td>Heidelberg</td>
<td>KST</td>
<td>KST</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KNeST</td>
<td>KNeST</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None⁴</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Typhimurium</td>
<td>ApiSspSuT</td>
<td>AKNePiSSpSuT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AnePiSSpSuT</td>
<td>AKSSpSuT*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AKNepiSSpSuT</td>
<td>ACpKNepiSSpSuT*</td>
</tr>
<tr>
<td>Herd C</td>
<td>Heidelberg</td>
<td>ND²</td>
<td>KNeST</td>
</tr>
</tbody>
</table>

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.

*Salmonella* Heidelberg and had PFGE patterns identical to the on-farm fecal isolates (data not shown).
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 \textit{Salmonella}, or fewer serovars, were present in feces compared to lymph nodes when pigs were necropsied on the farm.\textsuperscript{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\textsuperscript{13} showed that a 25-gram sample of feces is far superior to a 1-gram sample for \textit{Salmonella} detection. In addition, when pigs were necropsied on the farm and samples were subsequently submitted to a diagnostic laboratory for culture, \textit{Salmonella} isolates from the lymph nodes were the same as those from PPF samples of pigs in the same cohort.

Efforts to reduce \textit{Salmonella} rely on establishment of intervention strategies. The results of this study confirm the work of others,\textsuperscript{2–4} ie, we conclude that acute \textit{Salmonella} infection during transport and lairage requires intervention in order to control \textit{Salmonella} contamination of pork. If indeed pigs exposed to \textit{Salmonella} hours before slaughter can become systemically infected, pigs from \textit{Salmonella}-free sources, or sources with a low prevalence of \textit{salmonellae},\textsuperscript{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

- \textit{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 \textit{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 \textit{Salmonella}.
- The time immediately prior to slaughter, including both transport and lairage, should be considered a key intervention point for control of \textit{Salmonella} in pork production.

Acknowledgements

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References – refereed


References – non refereed