Title: Eliminating the Abattoir Pen Lairages to Decrease the Prevalence of Salmonella in Cull Sows - **NPB #02-129**

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Abstract: The objective of this study was to determine the role of the antemortem pens at an abattoir as a preharvest source of *Salmonella enterica* contamination. In this study, consisting of 4 sampling periods from February-April 2002, forty (40) sows were selected for each period at the same concentration point and transported to the abattoir. At the abattoir twenty (20) sows were unloaded and sent directly to harvest, the other 20 held in antemortem pen for 2 hours before harvest. Samples collected from the carcasses included ileo-cecal and, subiliac lymph nodes, cecal and transverse colon contents, pre-rinse carcass sponge swabs, utilizing USDA-FSIS protocol (300 cm^2), for the right and left carcass sections and a chopped meat block from these carcasses. Samples were screened by an enzyme-linked immunosorbent assay (ELISA), positive at OD value of 0.400 or greater, and confirmed by culture, isolation and identification of Salmonella colonies. The percentage of positive tissue samples from the cull sows held in the antemortem pens (59%) was significantly higher (P< 0.05) compared to the direct delivered sows (44%). Percent positive cecal contents from sows exposed to the antemortem pens (55%) were significantly higher P< 0.05) compared to cecal contents of direct delivered sows (39%). The study demonstrates that normal antemortem holding practices contributed to increased *Salmonella enterica* contamination of the digestive tract prior to harvest.

Introduction: Salmonella contamination of all classes of pork products is a growing concern for the pork industry. We have previously demonstrated that 40-70% of midwestern market swine are Salmonella positive after normal farm-direct transportation and antemorten holding. Cull sows are a greater potential risk for introduction of food borne Salmonella contamination, in part because of peri-marketing normal holding and handling practices. Sows can become infected with Salmonella either directly by feed consumption or animal contacts or by exposure to a contaminated peri-marketing environment. Since much of the pork derived from cull sows is handled as ground meats and used in processed products sold directly to consumers the risks of Salmonella contamination may be greater food-borne risk than in finished pork products. Few studies have examined Salmonella risk relationships under modern procurement and processing practices. This study was designed to examine the role of the abattoir antemortem pen in peri-marketing Salmonella contamination.

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Objectives: To determine the impact of abattoir holding pens on Salmonella prevalence in cull sows.

Materials and Methods: This study, completed over 4 sampling periods from February–April 2002, involved 160 cull sows handled under normal peri-marketing conditions. Buying station personnel, based on condition scores and weight limits set by the purchasing sausage company, selected cull sows from a larger population at a buying station. Forty sows selected each period were transported to the abattoir by commercial trucker. At the abattoir, twenty sows were unloaded from the truck and sent directly to harvest. The other twenty sows were held in pens for 2 hours, and then harvested under the same conditions. Each of the 40 study carcasses was individually identified and samples collected to maintain that identity. Carcass samples collected included ileocecal lymph node (5-10 g), subiliac lymph node (1-5 g), cecal contents (20 g), transverse colon contents (15 g), pre-carcass wash sponge swabs of the left and right carcass section utilizing the standard three-site USDA-FSIS procedure (300 cm^2), and chopped meat block sample from a mixture of each 20 sow group. All samples were placed in whirl-pak bags and transported on ice to the National Animal Disease Laboratory, Ames, Iowa.

In the laboratory, 10 g colon and cecal contents were placed in 90 ml of tetrathionate broth and GN-Hajna broth, two enrichment media. The subiliac lymph nodes from each carcass side were pooled, placed in a sterile bag, and macerated with a rubber mallet. The ileocecal lymph nodes were treated in the same manner in a separate bag. Ten ml of peptone water was added to each lymph node-containing bag and the sample homogenized for one minute in a stomacher at 260 rpm. One ml of supernatant was added to 9 ml of the two enrichment media. Carcass sponges were cut lengthwise into four sections; one section from each side was pooled and placed into 18 ml of each selective medium. All inoculated media were incubated overnight at 37 °C.

At 24 hours, the tetrathionate broth and GN-Hajna broth cultures were transferred (1:100) into Rappaport R-10 medium and incubated for 24 hours at 37 °C. After 24 hours, all rappaport tubes were screened by an enzyme-linked immunosorbent assay (ELISA). Samples with OD values 0.400 or greater were considered to be positive on the screening procedure. All tubes testing positive samples were streaked for colonies on xylosine-lysine-tergeted-4-agar (XLT-4) half plates and incubated overnight at 37 °C. One colony exhibiting typical *S. enterica* appearances were picked to Rambach agar and incubated overnight at 37 °C. Presumptive positive isolates were serogrouped and picked to tryptase soy agar (TSA) slants then submitted to the National Veterinary Services Laboratory, Ames, Iowa, for serotype determination.

Results: A total of 160 cull sows from a single buying station were selected and sampled over a 12-week period. Selected animals were handled identically in each period except for the antemortem holding period at the abattoir. Individual animal results were collected and analyzed but aggregated for reporting purposes. There was a statistical difference for cumulative sample types when compared between treatment (hold) and control groups (no hold) within the four sampling periods. Cecal contents isolation rates were statistically significant ($P < 0.05$). All others sample types were not significantly influenced by holding. However the cecal content values were sufficiently different to cause the cumulative values to be statistically different.

Table 1 contains the summary data for all carcass Salmonella isolations. The transverse colon was used as a surrogate sample for an antemortem fecal sample because of safety and logistical concerns associated with obtaining a pre-harvest sample in the available antemortem pen environment.

Table 1: Tissue percentage positive and number tested of sows held for two hours in a holding pen versus sows that were not held in a holding pen

	No Holding	Holding	P-value
Transverse colon	5%(80)	4%(80)	No Difference
Cecal contents	39%(80)	55%(80)	$P < 0.03$
Ileocecal Lymph Node	9%(80)	9%(80)	No Difference
Subiliac Lymph Node	0%(80)	1%(80)	No Difference
Carcass Swabs	1%(80)	0%(80)	No Difference
Meat Block*	0%(7)	0%(7)	No Difference
Overall[#]	44%(80)	59%(80)	$P < 0.05$

* Consisted of twenty sows sub sampled seven times
[#] Consisted of transverse colon, cecal contents, ileocecal and subiliac lymph nodes

Isolates from all positive cultures were submitted to National Veterinary Services Laboratory for serotype classification. In each sample period the pens demonstrated from one to three (3) serotypes. Serovars recovered from the pen were Reading (6), Derby (4), Uganda (2), Manhattan (2), Bovis-Morbificans (1), and Senftenberg (1). Tissue isolations were of a greater diversity and number than found in the pens. *S. enterica* serovars isolated from held sows (n = 61 isolates) were Derby (19), 6,7:z10-monophasic (15), Brandenburg (10), Infantis (6), Hadar (5), Johannesburg (4), and Tennessee (2). *S. enterica* serovars isolated from "no hold sows" (n = 49) were Brandenburg (16), Derby (12), Hadar (8), Infantis (6), Johannesburg (3), 6,7:z10-monophasic (3), and Typhimurium (1).

Discussion: The holding period of 2 hours in the antemortem pens was sufficient to increase the isolation rates in cecal contents but not in the other tissues sampled. The cecal contents appear to rise more quickly than other tissues when exposed to contaminated environments. This observation is consistent with findings in finishing swine. As expected, the level of intestinal contamination was relatively higher than in farm-direct market swine. This heightened contamination level may result from several interrelated factors. The cull sows selected had 18-24 hours of exposure to an environment (concentration point) where commingling with sows of other farms occurred. Therefore both direct and indirect exposure opportunities were possible. These longer transit periods from the farms to abattoir and continuous exposures to commingled environments offered recurrent opportunity for individual animals to become contaminated prior to abattoir delivery. This factor may partially explain the absence of significant differences in cecal content isolations when examined by test period, but a significant difference in the aggregate. The potential for increasing the cecal isolation rates by a significant amount over the two-hour holding period is decreased as the entering values rise. In addition weekly variations in the environmental contamination of the antemortem pens may have played a role in this rise.

The much lower isolation rates for the transverse colon as compared to the cecal contents and the lack of difference between direct and holding groups is somewhat surprising. The lack of difference between the holding and direct groups was not anticipated. However, this observation is consistent with a transient infection that has not had sufficient time to traverse the entire tract. The relatively low ileo-cecal isolation rates within and between treatment groups may result from a similar temporal or dose exposure function. Low-level environmental exposures may be sufficient to contaminate the cecal contents but may require higher dosages to consistently be taken into the gut-associated lymph system. Additional work is needed to further elucidate this observation.

The finding of minimal Salmonella isolations from the carcass-associated lymph system, as represented by the subiliac lymph nodes, is consistent with observations in market swine. As with the ileo-cecal nodes there may be a threshold exposure level necessary for colonization. It would be expected that such threshold levels would be higher than in the ileo-cecal lymph node. The minimal carcass surface (carcass swabs) and meat block contamination rates most likely represent the care in butchering and the HACCP controls implemented by the processing facility. Even though a substantial percentage of the gut tracts contained Salmonella it was not transferred from the gut to the carcasses during the butchering process.

The greater serotype diversity and frequency of isolation in the “hold” groups indicated that the antemortem pen was a likely contributor to increased Salmonella isolation rates. Three of the seven pen isolates were not found in the tissue samples, or at least were not identified in the culture colonies selected for classification. Whether they were represented on the initial culture plate and not selected, can’t be practically known under current procedures.

Lay interpretation: This project has demonstrated that the antemortem holding pens at an abattoir may play a role in increasing the number of Salmonella-infected cull sows entering the abattoir. It also provides an indication that other peri-marketing concentration and transport steps may be involved in this contamination, as exhibited by the heightened isolation rates of the direct harvested (no hold) groups when compared to levels expected in farm-direct marketed swine. Exposure to contaminated facilities for as short as a 2 hour period may raise these isolation rates. The cecal contents demonstrated the only significant rise in all the tissues cultured. This is consistent with other field and laboratory exposure observations. The failure to culture Salmonella from carcass swabs or the meat blocks produced from the test animals demonstrates that it is possible to reduce Salmonella contamination of meat products by implementing appropriate butchering techniques and other HACCP-based control steps at the packing plant level.

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