RESEARCH REPORT
PORK SAFETY

Title: Evaluation of Antemortem Salmonella Detection Procedures in Market Weight Swine – **NPB# 00-098**

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Abstract:

The objective of this study was to compare fecal culture, meat juice ELISA, and culture of abattoir collected tissues for their ability to accurately estimate the on-farm prevalence of *Salmonella enterica* in market swine. Six conventional commercial herds, depopulated in the Accelerated Pseudorabies Eradication Program (APEP) were used. One-half of study pigs (50) were necropsied on-farm; the other half (50) at a commercial abattoir. The true farm prevalence (TFP), based on the summation of positive cultures from ileocecal lymph nodes, cecal contents, or fecal samples was estimated at 5.3%. This estimate was higher than any provided by a single sample type cultured. The seroprevalence (meat juice ELISA) was estimated at 20% using a cutoff of OD% > 40. Swine were transported directly to the abattoir in disinfected trucks. They were allowed to reside in the antemortem pens for an average of 2.5 hours (1.5 to 3.5 hrs) before harvest. The tissues sampled were the same as on-farm. Prevalence estimates from abattoir collected samples were significantly higher than TFP; 39.9% of pigs were culture positive. Three *Salmonella enterica* serotypes were identified on-farm. These and an additional 14 serotypes were found in abattoir collected samples. This study shows that a single round of fecal collections will underestimate the true *Salmonella* status of a herd and that samples collected at the abattoir will overestimate the on-farm prevalence. It also demonstrates a significant rise in prevalence and serotype distribution between the production site and abattoir. Antemortem holding pens are considered a significant risk factor for this rise in prevalance.

Introduction:

Prior studies have reported that *Salmonella enterica* rates in market swine are three to ten times higher when measured after transport and harvest compared to on-farm rates (Berends et al., 1996). One suggested reason for this increased isolation rate is long term lairage (>12 hours) in contaminated abattoir holding pens. Another common rationale is that prevalence rises from a transportation stress reaction. However, such effects are inferred from indirect measurements. Isaacson et al (1999) reported increased isolation rates after transport only if pigs were not fasted before transport, and concluded that transport stress alone was not sufficient to increase isolation rates. In support of this conclusion, we demonstrated no increase in isolation rates after the stress of lairage (18 hours in a clean, disinfected facility), commingling or fasting (Hurd et al, 2001a)

It has been reported that carcass contamination after harvest is primarily related to intestinal *S. enterica* infections. The more *S. enterica* carried into the abattoir via the pig's intestine the greater the risk of equipment and final product contamination (Craven et al, 1982). In our lairage study (Hurd et al, 2001a) it was observed that pigs were becoming infected after leaving the farm although they were transported in clean, disinfected vehicles and held in antemortem pens for less than three hours. The physiologic feasibility of such rapid uptake has been reported by Fedorka-Cray et al (1995). In an experimental study using Nalidixic acid resistant *Salmonella enterica* serovar typhimurium, market weight swine could become infected after two hour exposure to contaminated floor (Hurd et al, 2001b). Identifying the source(s) of this contamination is important because it focuses control efforts where most effective interventions can be imposed. If abattoir holding pens are a primary source of contamination it may reduce the effects of individual on-farm *S. enterica* reduction efforts.

Objectives:

1. Estimate the sensitivity of on-farm collected fecal loops and ELISA serology by comparing on-farm collected fecal loops, cecal contents, and gut- and carcass-associated lymph nodes cultured by standard microbiologic techniques.

2. Compare on-farm collected fecal loops with cecal contents, and gut- and carcass-associated lymph nodes collected after transport and lairage.

3. Compare the distribution of serotypes found on the farm with those found after commercial transport and lairage in the slaughter house.

Procedures:

Six herds enrolled in the Accelerated Pseudorabies Eradication Program (APEP) provided a unique opportunity to test and necropsy a large number (600) of market weight pigs on the farm and at the abattoir. To avoid depression of market hog prices, all volunteering herds were to be depopulated; animals were to the euthanized and sent to rendering. Three days before the scheduled depopulation, study pigs were individually identified and a 1 g antemortem fecal sample was collected (AFEC). On the day of depopulation, the study pigs were randomly divided. One-half (50) were to be necropsied on-farm; the other half (50) at a commercial abattoir.

The pigs chosen to be necropsied at the abattoir were transported, along with others from the farm, in commercial vehicles that had been washed and disinfected. Only pigs from the depopulating farm were carried on these trucks. At the abattoir, study pigs were held together in a standard antemortem holding pen for about 2.5 hours. After stunning and exsanguination, they were diverted from the line for necropsy. Samples of the following were aseptically collected: ileocecal lymph nodes (ILC), ~10 g cecal contents (CC), superficial inguinal lymph nodes (SIL), and 1 g feces. Additionally, a ~10 g section of gluteal muscle was collected for the Danish mixed-ELISA analysis of the meat exudate. The 1 g necropsy fecal sample (NFEC) was collected, through the anal orifice, in the same manner as the antemortem fecal. The following day, pigs selected to remain on-farm were humanely euthanized. The same samples were collected in an identical manner as at the abattoir.

All samples were placed on ice and transported to the National Animal Disease Center (NADC, Ames, Iowa). The gluteal muscle sections were frozen (-20 C), and were later sent to the laboratory of Dr. D. L. Harris, Iowa State University, where the Danish ELISA was performed using a muscle exudate (meat juice). The remaining samples were refrigerated (40 F) until processed the following morning. For processing, the SIL nodes (10 g) and the ILC nodes (5 g), were separately macerated in a sterile bag with a rubber mallet. Peptone water[a] (10 ml) was added and each sample was homogenized using a stomacher at 260 rpm for one minute. One ml of supernatant was then added directly to each pre-enrichment medium (10 ml). Cecal and fecal samples (10 g) were added directly to each pre-enrichment broth (100 ml). For *S. enterica* cultures, pre-enrichment included separate tubes of GN-Hajna broth[a] (24 hours @ 37 C) and tetrathionate broth[a] (48 hours @ 37 C) followed by enrichment in Rappaport-Vassiliadis media[a] (24 hours @ 37 C). A portion of the Rappaport-Vassiliadis media was then streaked to brilliant green sulfa (BGS) agar[a] (24 hours @ 37 C) and XLT4 agar[a] (24 hours @ 37 C) plates, after which a single suspect colony from each plate was picked and transferred to triple sugar iron[a] and lysine iron agar slants[a] (24 hours @ 37 C). Biochemically suspect isolates were further classified by agglutination using *Bacto S. enterica O antiserum Groups poly A-I&Vi, B, C1* and *E*.[a] These isolates were then placed on trypticase soy agar[a] slants and shipped to National Veterinary Services Laboratory (Ames, Iowa) for serotyping.

For analysis, a pig was considered positive if it was positive on any one of the sample types. The true farm prevalence (TFP) was defined as the *Salmonella* prevalence estimated when combining the culture results from all sample types. The relative sensitivity (RSE) for a particular sample type was calculated as the number of samples positive by that sample type divided by the number of pigs positive on any of the sample types.

Results:

Objective 1. Estimate the sensitivity of on-farm collected fecal loops and ELISA serology by comparing on-farm collected fecal loops, cecal contents, and gut- and carcass-associated lymph nodes cultured by standard microbiologic techniques. Table 1 shows the prevalence estimates and 95% confidence intervals (CI) provided by each of the sample types collected during on-farm necropsy. The estimate provided by the AFEC (1.1%, CI = 0% to 2.3%) was similar to that from the NFEC samples collected 4 days later (0.7%, CI = 0% to 1.7%). The prevalences were similar but came from different positive pigs. No SIL nodes were positive on-farm. The point estimate from ILC node culture (3.2%, CI = 1.1% to 5.2%) was three times higher than the necropsy fecal prevalence estimate, although not significantly different ($p<0.05$) than the AFEC and NFEC samples.

The relative sensitivity estimate (RSE) of the 1 g fecal sample collected at necropsy (NFEC) was low. On-farm, the NFEC only detected 13% (2 of 15) of infected pigs. It missed all 10 of the pigs with positive ICL nodes. When results from on-farm and abattoir necropsies were combined, the relative sensitivity of NFEC improved. The overall RSE of the NFEC was 57.4% (74 of 129). However, NFEC still only detected 12.2% (9 of 74) pigs with infected lymph nodes (ICL and SIL combined).

Objective 2. Compare on-farm collected fecal loops with cecal contents, and gut- and carcass-associated lymph nodes collected after transport and lairage. Figure 1 compares the mean *S. enterica* isolation rates (SIR) for all herds, by samples collected at the farm (FSIR) and at the abattoir (ASIR). This figure shows that the pig prevalence was seven times higher ($P<0.001$) for pigs that were necropsied after transport and holding; 5.3% versus 39.9%. The ASIR was significantly ($P<0.05$) higher for all sample types (NFEC, CC, LNN) as evidenced by non-overlap of 95% confidence intervals (Table 2). The LNN had the smallest rate difference (3.6% compared to 9.1%). Nevertheless, it was still statistically significant ($P<0.05$).

A few herd level factors were evaluated. Linear regressions showed no effect of herd size, transport distance or holding time on the rate differences for all sample types. Herd 1 was the only one that did not show a significant difference between FSIR and ASIR. Additionally, it was the only herd where the pigs were not alternately assigned to necropsy location. At the abattoir Herd 3 had a large number of *S. enterica* serovar Typhimurium variety copenhagen isolations (n=25). Nine of those 25 isolates were from LNN. Notably, this was the only herd with a significant difference in the isolation rates for LNN. This serovar was the most frequently isolated from lymph nodes in all herds.

Objective 3. Compare the distribution of serotypes found on the farm with those found after commercial transport and lairage in the slaughter house. Table 3 provides a comparison of serotypes found on-farm and at the abattoir by herd and

tissue sampled. For all tissues the on-farm isolation rates were significantly lower than comparable abattoir samples. The diversity of serotypes was substantially greater in all abattoir tissues. Serotypes found in no on-farm samples were cultured from abattoir samples. Herd 4 was the only farm to demonstrate the same serotype (*S. derby*) in on-farm and abattoir samples. In addition to this serotype an additional six (6) new serotypes were isolated at the abattoir.

The design of this study allows us to rule out variation in sample type collected as a reason for the larger differences observed in SIR between the farm and abattoir. The same tissues were collected at both locations. The only differences between groups were the method of euthanasia and the length of time dead before collection of samples. It is not expected that method of euthanasia would affect SIR. A regression analysis showed that length of time dead had no impact on SIR.

The serological data in this study suggests that most infections discovered at the abattoir were not due to recrudescence. Latently infected pigs should show serological evidence of current infection, as measured by the Danish ELISA or some other valid serological test. Additionally, many latently infected pigs should be detectable by culture of lymph nodes during on-farm necropsy. All seroprevalence rates are lower than ASPR. Unless these herds were experiencing an acute outbreak of *S. enterica* or the ELISA test is faulty, many of the infections observed at the abattoir were newly acquired after leaving the farm. The data from Herds 2 and 6 support the possibility of recrudescence. For Herd 6, the seroprevalence and the ASPR are similar. In Herd 2, the seroprevalence is much higher than the ASPR. While this culture prevalence could have resulted from recrudescence, the variety of additional serovars recovered at the abattoir suggests non-farm sources of *S. enterica* infection.

Therefore, the remaining and most likely explanation for the large increase in *S. enterica* isolation rates, farm to abattoir, is rapid infection during transport and particularly during holding. Rapid infection among herd-mates could be occurring during transport. Study pigs were transported along with other swine from the farm, including breeding stock and younger pigs. This possibility is particularly evident in Herds 4 and 5, where there is an increase of farm resident serovars. However, in all test herds additional serovars, not found on-farm, were also recovered from the abattoir-collected samples. Additionally, isolates of the same serovar may actually be different clones, indicating a non-farm source. Further characterization of these isolates is needed to address this question.

All except Herd 1 demonstrate *S. enterica* acquired from non-farm sources. Transport trailers were disinfected. Therefore, holding pens remain a probable source. A recent publication and our non-published data demonstrate high levels of *S. enterica* contamination in abattoir holding pens. The weight of evidence suggests that rapid infection, particularly in holding pens, is the major cause of increased *S. enterica* isolation from market swine. This finding identifies an important *S. enterica* critical

control point in the pork production chain. Further research should focus on interventions to reduce the risk from holding pens.

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Table 1. Description of herds from which market swine were necropsied for *Salmonella enterica* isolation.

Herd Number	**Date Depopulated**	**Herd size (~# sows)**	**Holding Time (hrs)**	**Housing of market swine**
1	01/27/00	450	3.25	Total confinement, fully slatted floors
2	03/08/00	170	2.5	Outside lots[1], concrete or dirt floors
3	03/21/00	30	2.5	Outside lots[1], concrete or dirt floors
4	03/29/00	214	2.0	Outside lots[1], concrete or dirt floors
5	04/04/00	125	3.0	Total confinement, fully slatted floors
6	04/12/00	168	1.5	Partial confinement[2], partial slatted floors
Mean		193	2.5	

[1]Outside lots included open front buildings
[2]Partial confinement. Some pigs were housed indoors others in outside lots.

Table 2. *Salmonella enterica* isolation rates and seroprevalence for pigs necropsied on-farm or at the abattoir. Number of samples tested are shown in parenthesis. For each herd, percentages with the same superscript are significantly different between farms.

Sample type	Ante-mortem fecal[1]	Necropsy fecal			Cecal contents			Lymph nodes[2]			Pig prevalence[3]			Meat juice ELISA OD% >=40		
Collection site	Farm	Farm	Abattoir	Rate difference[4]	Farm	Abattoir	Rate difference[4]	Farm	Abattoir	Rate difference[4]	Farm	Abattoir	Rate difference[4]	Farm	Abattoir	Combined farm and abattoir
Herd 1	1.0% (97)	0.0% (48)	2.0% (50)	2.0%	0.0% (48)	2.0% (50)	2.0%	6.3% (48)	6.0% (50)	-0.3%	6.3% (48)	10.0% (50)	3.80%	0.0% (48)	0.0% (45)	0.0% (93)
Herd 2	1.1% (94)	0.0% (44)	30.0% (50)[a]	30.0%	0.0% (44)	4.0% (50)	4.0%	4.5% (44)	0.0% (50)	1.5%	4.5% (44)[h]	38.0% (50)[h]	33.5%	77.3% (44)	58.0% (50)	67.0% (94)
Herd 3	0.0%	0.0% (45)[b]	36.4% (44)[b]	36.4%	0.0% (45)[e]	13.6% (44)[e]	13.64%	2.2% (45)[g]	27.3% (44)[g]	25.1%	2.2% (45)[i]	54.5% (44)[i]	52.3%	0.0% (44)	0.0% (42)	0.0% (87)
Herd 4	4.4% (90)	4.4% (45)[c]	31.1% (45)[c]	26.7%	4.4% (45)[f]	53.3% (45)[f]	48.89%	4.4% (45)	0.0% (45)	-4.4%	8.9% (45)[j]	68.9% (45)[j]	60.0%	13.3% (45)	11.1% (45)	12.2% (90)
Herd 5	2.0% (98)	0.0% (49)[d]	51.0% (49)[d]	51.0%	6.1% (49)	10.2% (49)	4.08%	4.1% (49)	10.2% (49)	6.1%	10.2% (49)[k]	61.2% (49)[h]	51.0%	25.0% (48)	21.3% (47)	23.5% (95)
Herd 6	0.0% (98)	0.0% (50)	2.1% (48)	2.1%	0.0% (50)	2.1% (48)	2.08%	0.0% (50)	6.3% (48)	6.3%	0.0% (50)[l]	10.4% (48)[l]	10.4%	8.0% (50)	6.3% (48)	7.1% (98)
Mean (total)	1.47% (566)	0.7% (281)	25.2% (286	24.5%	1.8% (281)	13.6% (286)	11.9%	3.6% (281)	9.1% (286)	5.53%	5.3% (281)	39.9% (286)	34.52%	20.0% (280)	17.0% (286)	18.55% (557)
95% confidence limits (lower ,upper)	0%-2.3%	0%-17.%	20.1%-30.2%		0.2%-3.3%	9.7%-17.6%		1.4%-5.7%	5.8%-12.4%		2.7%-8.0%	34.2%-45.5%		15.3%-24.7%	12.5%-21.4%	15%-22%

[1]1 g fecal sample taken 3 days before depopulation.
[2]Results from the ileocecal and superficial inguinal lymph nodes are combined.
[3]Pig prevalence is defined as *S. enterica* isolation form any of the samples collected at necropsy (1 g fecal, cecal contents, or lymph nodes).
[4]Rate difference = abattoir percentage minus farm percentage.

Table 3. *Salmonella enterica* serovars (number of recoveries) from tissues collected at farm and abattoir necropsy for six swine herds.

Herd Number	Necropsy Fecal		Cecal Contents		Lymph Nodes		Antemortem Fecal
	Farm	Abattoir	Farm	Abattoir	Farm	Abattoir	
1		TYC (1)		TYC (1)	TYC (2) AGN (1)	TYC (2) AGN (1)	none typeable
2		DER (5) INF (2) RDG (4) TYC (1)		DER (1)	CHK (2)	CHK (3)	none typeable
3		BOV (1) AGN (1) DER (1) MUS (1) TYC (12)		TYC (4)	MAN (1)	TYC (9) AGN (1) BOV (1)	no isolates
4	DER (2)	DER (3) HEI (2) LIL (1) SPA (1) MAN (1)	DER (2)	DER (19) OHI (1) TYC (2	DER (2)		DER (3)
5		DER (2) MUS (1) MUC (16) LON (1) MVD (3)	LON (2)	MUC (2) MVD (2) OHI (2)	MUC (1) TYC (1)	DER (2) MUC (3) HEI (1)	HEI (1) LON (1)
6		MUC (1)		none typeable		AGN (2) CHK (1)	no isolates

AGN = Agona, ANA = Anatum, BOV = Bovis morficans, CHK = Cholerasuis var. kuzendorf, DER = Derby, HEI = Heidelberg, INF = Infantis, LIL = Lille, LON = London, MAN = Manhattan, MUS = Muenster, MUC = Muenchen, MVD = Montevideo, OHI = Ohio, RDG = Reading, SPA = St Paul, TYC = S. typhimurium