RESEARCH REPORT
PORK SAFETY

Title: Evaluating the sources of Salmonella after carcass chilling – NPB #10-146 revised

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Industry Summary

This study evaluated several aspects of salmonella contamination of pork after slaughter but prior to retail. First, a comparison was made between the USDA-FSIS carcass swab procedure and two other sampling methods. The FSIS carcass swab had the lowest level of sensitivity in comparison to excision samples and the M-Vac sampling device. This suggests that the carcass sponge swab method may be providing less accurate results, leading processor to miss early signs of processing deviations. A second comparison was made between the prevalence of salmonella as determined by carcass swabs, loin swabs and trim samples in a commercial establishment. The data shows that salmonella on finished carcasses was rare, but when it did occur, it appeared to occur frequently within carcasses from the same herd. A higher incidence on carcasses was also reflected in positive loin samples. Finally, a series of experiments were conducted to evaluate the potential for conveyor belts to be a potential source of cross contamination. These studies showed that, irrespective of belt material type, conveyor belts could be a potential source of cross contamination, even at low populations.

Keywords:

salmonella, chilled carcasses, contamination; conveyor belts; carcass sampling

Scientific Abstract:

A comparison was made between three carcass-sampling methods for Biotype I Escherichia coli. The methods included the USDA-FSIS sponge swab method, a surface excision method, and the Microbial-Vac Systems M-Vac sampler. There was a statistically significant difference between the methods, in the recovery of inoculated E. coli on pork carcasses, with the M-Vac recovering the highest populations, followed by the excision and swab sampling. Carcass, loin and trim from loins were sampled in a commercial processing establishment for the qualitative presence of salmonella. On three of the four sampling visits, a total of 2 out of 1180 samples were positive for salmonella (1 carcass and 1 trim). There were 25 positives out of 60 samples from the fourth visit (16 carcasses and 9 loins). This suggests that the level of salmonella on the incoming animals is highly variable, and

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that on any given day a number of carcasses may be positive. Finally, a series of experiments were conducted to evaluate the potential for conveyor belts to be a point of cross contamination for fabricated pork. Pork loins were inoculated with salmonella at a high and low population, and placed on to samples of three different conveyor belts. After 1 minute of contact, the inoculated loins were removed, and an un-inoculated loin placed on the conveyor belt sample. Both high and low inoculum loins transferred sufficient salmonella to the conveyor belt samples to result in transfer to the uninoculated loins.

Introduction:

Regulators and consumers are interested in pathways to contamination but currently much of this information is proprietary and there is a vacuum of publically available information and consequently many questions are unanswered. We propose a study design to use “semi-simulated” fresh ground pork that will make such post-harvest information publically available. The availability of “semi-simulated” ground pork information can be incorporated into our companion risk assessment and therefore consumer, industry and government groups can utilize the information to make better risk-informed decisions about Salmonellae control.

Understanding the methods of introduction of Salmonellae into pork has been the focus of intense research for many years and one of the remaining perplexing questions is pathways for introduction of Salmonellae in fresh ground pork (prior to retail). There are several proposed pathways for introduction of Salmonella into fresh ground pork product and this project aims to provide empirical data to support or refute these.

Alternative pathways for introduction of Salmonellae into fresh ground pork are contamination during the fabrication process and/or poor correlation between the FSIS estimate and meat contamination. With respect to the both these factors again very little is known as there is little information published about post-chilling levels of Salmonellae in pork products.

In a review conducted in 2008- 2009 to describe points of introduction and reduction of Salmonellae in pork, members of our group reviewed Salmonellae prevalence at points of pork from slaughter to fresh-shipped product. To be considered relevant for the review, studies had to report Salmonellae at more than one point in the production process. The 1st part of the review evaluated processing points from slaughter to cooler and after screening 5116 citations, 40 studies were identified that evaluated the presence of *Salmonellae* on pork carcasses during primary processing.[13, 14] The review provided little evidence that *Salmonellae* is introduced into the pork as it moves along the processing chain to the cooler. On the contrary, the aggregated data across the studies suggested that overall the processes employed from slaughter to the cooler are associated with

steady decreases in *Salmonellae* spp. prevalence (Figure 1)

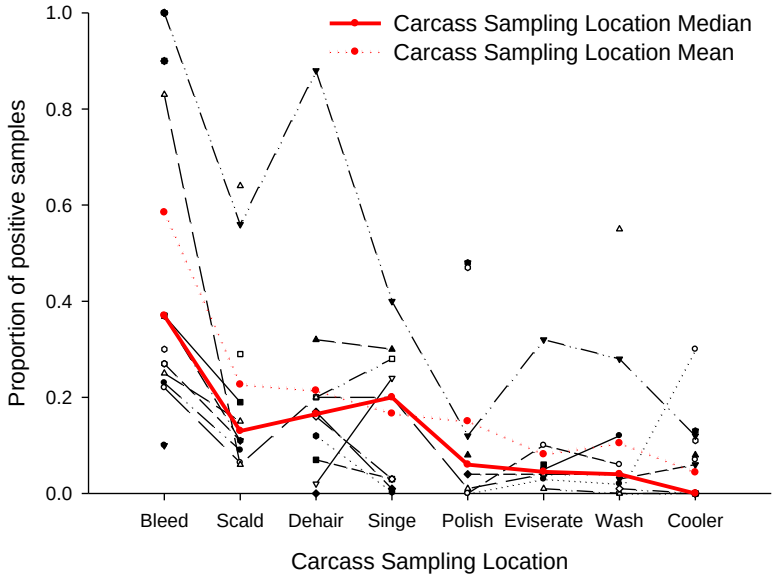

Figure 1:Line and scatter plot describing the proportion of *Salmonellae* positive samples for 40 studies and the median and mean of the studies at each carcass sampling location.

The 2nd part of the 2008-2009 review, which aimed to examine points of introduction or reduction of Salmonellae post chilling of the carcass, would be most relevant to the question of Salmonellae prevalence in lymph nodes or factors associated with sources of Salmonellae in fresh ground pork (prior to retail) however, that review was unable to identify publically available information to support or refute the hypothesis that either cross-contamination or deep-systemic lymph nodes were a source of Salmonellae in product after carcass chilling. [13] [14] The review staff looked in many locations for data about the topic to ensure as comprehensive assessment of the publically available information as possible : Agricola, CAB Abstract, AGRIS, MEDLINE, BIOSIS, Food Science Technology Abstracts (FSTA) Retrospective, Biological abstracts, Biological & Agricultural Index and FSTA. The tables of contents from the following conferences were also searched; The International Symposium on Epidemiology and Control of Salmonella in Pork (1996-2005), International Pig Veterinary Society (1969-2006), American Association of Swine Veterinarians/ Practitioners (1970- 2006) and The Annual Reciprocal Meat Conference (1999-2006).

From 713 references identified during the literature search, only four manuscripts describing 12 studies reported evaluating *Salmonella* spp. prevalence at multiple points in the post-cooler production process. Only two manuscripts were available in English that described sampling of pork after chilling at more than one location post chilling. Two studies also reported the prevalence of *Salmonella* spp. on carcasses and then at one sampling point in the processing chain after the cooler. None of these studies described the stages of processing employed by the study plants. Several potentially relevant articles could not be located and a large number of articles that appeared to be relevant were excluded because they were not published in English. There are obviously studies that report Salmonella at only one point post prevalence that were not considered by that review, however the review experience does re-iterate the paucity of publically available information about the post-chilling production that has previously been noted. Berands et al (1997) [15] noted that information about cutting plants was rare and “practically all of which is published in confidential reports in Dutch or in specialized books of limited circulation”. At the time of preparing the NPB and AMIF report the

reviewers acknowledged that it is possible that the required information is available but subject to confidentiality agreements. The impact of the paucity of available data is that empirical evidence of efficacy of control programs in the post-cooler side of pork processing is not available publically and therefore consumers and regulators still question the control of Salmonellae post-harvest.

This project aims to address that vacuum of data, so that information can be incorporated into our companion risk assessment and therefore consumer, industry and government groups can utilize the information to make better risk-informed decisions about Salmonellae control.

Objectives:

Objective 1: Determine the incidence of salmonellae in pork trim
Objective 2: Assess the efficacy in recovering bacteria from pork carcasses using multiple sampling methods
Objective 3: Determine the incidence of salmonellae from edible portions of the pork carcass
Objective 4: Determine the potential for cross contamination by salmonellae during fabrication of pork carcasses

Materials & Methods:

Objective 1: Determine the incidence of salmonellae in pork trim

Trim samples from fabricated loins from previously sampled carcasses and loins (see Objective 3) were collected from the loin trimming line. The trim samples were collected from loins fabricated 50 carcasses each from two separate herds. On each visit, 10 composite trim samples were collected from each group of 50 fabricated loins. On each sampling visit, trim from loins from 50 carcasses from each of two different herds (100 carcasses total) were sampled, and the processing establishment was sampled weekly over four consecutive weeks (representing 400 carcasses total).

Microbiological Methods: A 25 – 30 gram sample was prepared from each composite trim sample by manually shredding the each trim sample with a sterile knife. Samples were analyzed using the Neogen AN SR PCR system. Briefly, the samples were enriched in ANSR enrichment broth 2 for 18-24 hours at 42^{o}C. 50 μl of the enriched sample was mixed with 450 μl of lysis buffer and heated at 80^{o}C for 20 minutes. 50 μl of the lysed sample was transferred to tubes containing the lyophilized reagents at 56^{o}C, and the samples evaluated in the ANSR reader over a 10 minute period. Results were recorded as positive or negative based on the manufacturer's software.

Objective 2: Assess the efficacy in recovering bacteria from pork carcasses using multiple sampling methods

The purpose of this study is to determine if poor sensitivity of the FSIS was one potential reason for a disconnect between the FSIS carcass swab measures of Salmonella and other measures post carcass swabbing. The revised project objective will be to compare the relative sensitivity of the FSIS method versus the M-vac method (Microbial-Vac Systems,Inc, Jerome ID; http://www.m-vac.com/) .

Pork carcasses were surface inoculated with a five strain mixture of non-pathogenic Escherichia coli bacteria (ATCC accession numbers BAA-1428, BAA-1429, BAA-1430, BAA-1431 and BAA-1432). From each inoculated area, a 100 cm^2 carcass swab was taken following the USDA-FSIS protocol, a 100 cm^2 excision sample was aseptically collected, and a 100 cm^2 area was sampled using the M-Vac microbial sampler. The samples were serially diluted and analyzed using the 3M Petrifilm E. coli/Coliform count plates (http://solutions.3m.com/wps/portal/3M/en_US/Microbiology/FoodSafety/product-information/product-catalog/?PC_7_RJH9U523003DC023S7P92O3O87000000_nid=C0WJ62882Vbe29BDXSBJ7FgI), following the manufacturer's instructions.

Objective 3: Determine the incidence of salmonellae from edible portions of the pork carcass

Carcass and loin swabs were collected from a commercial processing establishment. Carcass swabs were obtained after a minimum of 12 hours cooling using the standard FSIS methodology for carcass swabs. Sterile sponge swabs (Nasco, Janesville WI) were moistened with 20 ml buffered peptone water. An un-delimited area of at least 100 cm^2 was swabbed on each sampling location (ham, belly and jowl). Five carcasses were pooled into a single swab. On each sampling visit, 50 carcasses from each of two different herds (100 carcasses total) were sampled, and the processing establishment was sampled weekly over four consecutive weeks (400 carcasses total).

The loins fabricated from the same carcasses were sampled using moistened sterile sponge swabs. The loins were swabbed on the interior (cavity) surface, by using the narrow end of the sponge (2.54 cm width) the entire length of the fabricated loins. The loins were separated by herd, and five loin samples were pooled into a single composite sample. On each sampling visit, 50 fabricated loins from each of two different herds were sampled.

Microbiological Methods: The carcass and loin swabs were analyzed qualitatively for the presence of salmonella, using the Neogen Reveal 2.0 lateral flow ELISA test (Neogen, Lansing, MI). Briefly, the sponge swabs were diluted 1:10 and pre-enriched in buffered peptone water for 18-24 hours at 42^oC. 0.1 ml of the pre-enriched sample was transferred to RV broth (BBL) and incubated for 18-24 hours at 42^oC. The analysis using the lateral flow ELISA was conducted following the manufacturer's instructions.

Carcass sponge samples were also evaluated quantitatively for populations of *Enterobacteriaceae*. Briefly, 1.0 ml was transferred from the sponge samples prior to dilution with buffered peptone water and enumerated in duplicate on PetriFilm

Objective 4: Determine the potential for Cross Contamination by salmonellae During Fabrication of pork carcasses

Pork loins were obtained from a local retail establishment. The loins were surface inoculated with a 5 strain mixed culture of salmonella to an initial population of approximately 100 (low) or 10,000 (high) colony forming units/ 100 cm^2. The loins were placed on sections (ca. 45 cm x 45cm) of three different types of conveyor belts for 1 minute. A second, un-inoculated pork chop was then placed on the same area of the conveyor belt. Surface swab samples were obtained from the contact surface of

the un-inoculated pork loin and enumerated on non-selective agar. For the low inoculum level, samples were enriched and the presence of salmonella was determined qualitatively.
The three conveyor belt samples were (a) Ronanyl DM 8/2 A2+04 Light Blue Thermoplastic polyurethane (b) Volta FrMB-3.0 Blue CEB Thermoplastic elastomer blue and (c) Ropanyl DM Thermoplastic polyurethane 04+04 White food grade Amerol. The conveyor belt samples were sanitized between each replication by immersion in a minimum 1000 ppm free available chlorine and then extensively rinsing with distilled water.

Results:

Objective 1: Determine the incidence of salmonellae in pork trim

Objective 3: Determine the incidence of salmonellae from edible portions of the pork carcass

The sampling for these two objectives were combined for efficiency and to minimize disruption at the processing establishment. The data from these experiments are summarized in Table 1.

Objective 2: Assess the efficacy in recovering bacteria from pork carcasses using multiple sampling methods

The results of the different sampling methods are presented in Table 2. The data were analyzed using co-variate analysis, with the initial inoculum as the independent co-variate. Each method was statistically different ($P<0.05$) from the other, with the M-Vac recovering the highest numerical population from the carcass.

Objective 4: Determine the potential for Cross Contamination by salmonellae During Fabrication of pork carcasses

The results of the evaluation of potential cross contamination are presented in Table 3. There were no statistical differences between belt types ($P>0.05$) with the high inoculum, although this is in part attributable to the very low numerical results seen universally with replication 3. However, if the results from replication are eliminated from the analysis, there are still no statistically significant difference ($P>0.05$) between the belt types. The un-inoculated loins all tested positive for salmonella with the low inoculum levels, suggesting that even low levels of salmonella may be transferred by direct contact on the conveyor belts.

Discussion:

Objectives 1 and 3: Carcass, loin and trim from loins were sampled in a commercial processing establishment for the qualitative presence of salmonella. On three of the four sampling visits, a total of 2 out of 1180 samples were positive for salmonella (1 carcass and 1 trim; Table 1). There were 25 positives out of 60 samples from the third or four sampling visits (16 carcasses and 9 loins). This suggests that the level of salmonella on the incoming animals is highly variable, and that on any given day the level of incoming salmonellae may be sufficient to overwhelm the interventions commonly

used in commercial pork processing, resulting in a number of positive carcasses. This same event is seen in the processing of other production animal species, and is frequently referred to “hot days”.

Given the high variability of Salmonella isolation, estimates of the mean prevalence will have a wide confidence and not be very informative. It is likely much more data are needed for a reliable prevalence estimate. Further, given this wide variation and the number of low salmonella prevalence day, mean contamination may not be the most useful measure of effective interventions. Consideration should be given to trying to understand the frequency of “hot days”, interventions could then be assessed with respect to reduction of “hot days”.

Objective 2: A comparison was made between three carcass sampling methods for Biotype I Escherichia coli. The methods included the USDA-FSIS sponge swab method, a surface excision method, and the Microbial-Vac Systems M-Vac sampler. There was a statistically significant difference between the methods, in the recovery of inoculated E. coli on pork carcasses, with the M-Vac recovering the highest populations, followed by the excision and swab sampling. Although the actiual differences are small, and the inoculated populations much higher than would normally be expected, this may suggest that the relative sensitivity of the standard USDA-FSIS swab is lower than other methods. A lower sensitivity may result in many samples where the results are below the detection limit of the assay. While these results may appear reassuring to the processor, they may be providing an incomplete picture of the actual levels of contamination on the carcasses. This would result in a processor not recognizing a developing issue until after it had reached some level above the level of sensitivity of the method. This may in part account for the sudden appearance of the unexpected contamination events (“hot days”).

This information is very informative for a risk model evaluating the source of ground pork contamination. It suggests there is a “disconnect” betweed FSIS reported data on carcasses and the true prevalence. This “disconnect” can more accurately be described as poor sensitivity.

Objective 4. The series of experiments conducted to evaluate the potential for conveyor belts to be a point of cross contamination for fabricated pork found little evidence of belt differences but evidence of cross-contamination . Pork loins were inoculated with salmonella at a high and low population, and placed on to samples of three different conveyor belts.. Both high and low inoculum loins transferred sufficient salmonella to the conveyor belt samples to result in transfer to the un-inoculated loins.

These findings suggest “belt contamination events” can and likely do impact the Salmonella status subsequently processed loins and other cuts. This information is also informative for a risk model evaluating the relative impact of contamination source on final ground pork.

Table 1. . Summary of carcass samples from processing establishment..

Sampling Day[A]	Carcass[B]	Loin	Trim	Total	Carcass Enterobacteriaceae[C]
1 – A	1/10	0/10	0/10	1/30	3.13
1 – B	0/10	0/10	0/10	0/30	3.22
2 – A	0/10	0/10	0/10	0/30	2.79
2 – B	0/10	0/10	0/10	0/30	2.89
3 – A	10/10	4/10	0/10	14/30	3.14
3 – B	6/10	5/10	0/10	11/30	2.60
4 – A	0/10	0/10	0/10	0/30	2.82
4 – B	0/10	0/10	1/10	1/30	2.58
Total					

A Sampling day – Herd A or B

B Number of salmonella positive samples out of the total number of composite samples. For carcass and loin samples,, each composite sample represents 5 carcasses or loins. Trim samples were gathered at random from loins from each herd.

C Log_{10} colony forming units/cm^2

Table 2. Comparison of different carcass sampling methods. Covariate analysis with inoculum level as the independent variable.

Sample Method	Number of Samples	Arithmetic Average[A]	Logarithmic Average
Swab	22	590,798 (424,585)	5.60 (0.46)
Excision	22	742,409 (535,103)	5.72 (0.45)
M-Vac	22	1,029,704 (749,413)	5.84 (0.46)

A Mean(stand deviation); average colony forming unts/100cm^2

B Means with different superscripts are significantly ($P<0.05$) different.

Table 3. Cross contamination from inoculated pork loin to inoculated pork loin by conveyor belt.

Replication	Inoculum(cfu/100cm^2)	Belt type[A]		
		Belt 1	Belt 2	Belt3
1	100	Positive	Positive	positive
	10,000	2240	1055	355
2	100	Positive	Positive	positive
	10,000	7250	3550	200
3	100	Positive	Positive	positive
	10,000	105	195	200
Average	100	3/3	3/3	3/3
Average[B]	10,000	3,198 ($\log_{10}$ 3.08)	1,600 ($\log_{10}$ 2.95)	252 ($\log_{10}$ 2.38)

A: Belt 1: Ronanyl DM 8/2 A2+04 Light Blue Thermoplastic polyurethane; Belt 2: Volta FrMB-3.0 Blue CEB Thermoplastic elastomer blue; Belt 3: Ropanyl DM Thermoplastic polyurethane 04+04 White food grade Amerol

B: Least Squares Mean

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