RESEARCH REPORT
PORK SAFETY

Title: INTERVENTIONS TO CONTROL SALMONELLA I 4,[5],12:I:- IN PORK – **NPB #18-049**

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Industry Summary:
Several products made from pork trim are currently being sampled by USDA FSIS under their Raw Pork Products Sampling Program Phase II (USDA FSIS Notice 30-17). This proposal intends to evaluate the impact of commercially viable interventions on reducing non-typhoidal *Salmonella* on pork trim and on ground products made from pork trim. In addition, it will evaluate sampling plans and provide statistical comparisons between the plans, in regard to their abilities to provide reliable information to processors. In 2015, there was an outbreak of Multidrug-Resistant *Salmonella* I 4,[5],12:i:- infections linked specifically to roaster pigs, with 134 confirmed cases (see https://www.cdc.gov/salmonella/pork-08-15/). All of the pigs were processed at the same federally inspected establishment in Washington State. The establishment would be classified as "very small" by USDA Food Safety and Inspection Service (FSIS), and although they were following standard industry practices, they were not using any microbial interventions. It was suggested by several individuals at both USDA FSIS and the Centers for Disease Control that perhaps this serovar was unusually heat resistant, which is why it survived cooking and caused the outbreak. Alternately it was suggested that the bacterium could have been located in the lymph nodes, and this combined with a hypothetical increased heat resistance could have contributed to the outbreak.

The research reported here demonstrates there is no evidence to suggest that *Salmonella* serovar I 4,[5],12:i:- presents a new or unique challenge to fresh pork. The responses of a mixed culture of different isolates of *Salmonella* serovar I 4,[5], 12:i- was evaluated for susceptibility to carcass interventions, survival during refrigerated storage and thermal resistance after refrigerated storage. There were no observed differences in the response of a mixed culture of different isolates of *Salmonella* serovar I 4,[5], 12:i- when compared to mixed culture of reference *Salmonella* serovars. Based on these studies, there is no evidence to suggest that *Salmonella* serovar I 4,[5],12:i:- presents a new or unique challenge to fresh pork.

Key Findings:

- The response of *Salmonella* serovar I 4,[5],12:i:- to commonly used pork carcass interventions was not different from the response of common *Salmonella* serovars. There is no evidence to suggest that *Salmonella* serovar I 4,[5],12:i:- presents a unique challenge to pork carcass decontamination.
- The survival of *Salmonella* serovar I 4,[5],12:i:- during refrigerated storage in ground pork was not different from the survival of common *Salmonella* serovars. There is no evidence to suggest that *Salmonella* serovar I 4,[5],12:i:- presents a unique challenge during the refrigerated storage of pork.

- The heat resistance of *Salmonella* serovar I 4,[5],12:i:- during refrigerated storage in ground pork was less than the heat resistance of common *Salmonella* serovars. There is no evidence to suggest that *Salmonella* serovar I 4,[5],12:i:- presents a unique challenge during cooking of pork, and does in fact suggest that *Salmonella* serovar I 4,[5],12:i:- may be less of a risk than the common *Salmonella* serovars.
- The heat resistance of both common *Salmonella* serovars and *Salmonella* serovar I 4,[5],12:i:- decreased during refrigerated storage. All of the *Salmonella* serovars evaluated in this study were less heat resistant after 14 days storage at 5°C.
- ***General Conclusion***: Based on these studies, there is no evidence to suggest that *Salmonella* serovar I 4,[5],12:i:- presents a new or unique challenge to fresh pork.

Keywords: *Salmonella* serovar I 4,[5],12:i:-; pork carcasses; interventions; refrigerated storage; heat resistance

Scientific Abstract: A mixed culture of different isolates of *Salmonella* serovar I 4,[5], 12:i:- were compared to a mixed culture of reference *Salmonella* serovars. Two groups of *Salmonella* were compared for their resistance to commonly used pork carcass interventions, survival in ground pork and thermal resistance in ground pork. There were no observed differences between the response of the two different groups of *Salmonella* serovars within intervention type. In addition, non-pathogenic *Escherichia coli* surrogates were also evaluated, and there were no observed differences between the responses of the two different groups of *Salmonella* serovars and the non-pathogenic *E. coli* surrogates within intervention type. There were no observed differences in the recovery and survival of the two different groups of *Salmonella* serovars in ground pork which had been treated with interventions, ground and stored at 5°C for two weeks. Finally, there were no observed differences in heat resistance between the two different groups of *Salmonella* serovars in ground pork which had been treated with interventions, ground and stored at 5°C for two weeks. However, there were observed differences in heat resistance in both groups of *Salmonella* serovars associated with refrigerated storage. The heat resistance of both of the two different groups of *Salmonella* serovars decreased after refrigerated storage. The results of these experiments demonstrate that there are no observed differences between the responses of *Salmonella* serovar I 4,[5], 12:i:- when compared to the reference *Salmonella* serovars .

Introduction: *Salmonella* serovar I 4,[5], 12:i:- has been become the organism of interest for CDC due to a recent major outbreak associated with roaster pigs in the north-western states. In 2015, there was an outbreak of Multidrug-Resistant *Salmonella* I 4,[5],12:i:- infections linked specifically to roaster pigs, with 134 confirmed cases (see https://www.cdc.gov/salmonella/pork-08-15/). All of the pigs were processed at the same federally inspected establishment in Washington State. The establishment would be classified as "very small" by USDA Food Safety and Inspection Service (FSIS), and although they were following standard industry practices, they were not using any microbial interventions. Subsequent environmental sampling found the specific *Salmonella* strain in the lairage pens of the establishment. During the investigation, the chefs cooking pigs contended that the meat was cooked to appropriate temperature raising the suggestion that the organism might have been "resilient" to normal pathogen reduction processes and temperature. However, previous research (FAO/WHO 2016) suggests that current pork slaughter practices are effective in controlling non-typhoidal Salmonella.

Research published by Burns et al (2016) indicated that there was strain to strain variation in heat resistance between 5 strains of *Salmonella* serovar I 4,[5], 12:i:- when measured in trypticase soy broth, but concluded "overall the strains investigated do not appear to be that much more heat resistant than *Salmonella* previously studied." This study was primarily focused on pig feed, and not pork meat. There is no current evidence at present to suspect that this particular *Salmonella* serovar I 4,[5], 12:i:- which has only recently been "named" and which is a unique form of *Salmonella* Typhimurium. However, our project aims to address this possibility. There is a higher than expected level of *Salmonella* serovar I 4,[5], 12:i:- in submissions to the Iowa State University Veterinary Diagnostic Laboratory (VDL). These results are not published yet, however the results suggest that

Salmonella serovar I 4,[5], 12:i:- is frequently isolated from swine submitted to VDL at levels much higher than reported in the NARMS retail pork surveys. This latter unpublished finding suggests that *Salmonella* serovar I 4,[5], 12:i:- is likely on farms (as it is in sick pigs) and by extension it is therefore likely in the lairage of abattoirs, as the lairage holding pens are a" "mixing pot" for *Salmonella* {Schmidt, 2004 #3755}and likely expose all animals entering the plant to many organisms that they did not encounter at the farm of origin.

Objectives:

1. Determine the impact of commercially available antimicrobial interventions on the survival of non-typhoidal Salmonella, including Salmonella I 4,[5],12:i:-, on pork trim.

2. Determine the survival and recovery of *Salmonella* serovar I 4,[5],12:i:- in ground pork made from intervention treated pork trim.

3. Determine the heat resistance of Salmonella I 4,[5],12:i:- in ground pork treated with interventions.

Materials & Methods:

Bacterial cultures: A mixed culture of common *Salmonella* serovars was prepared for as a reference culture. All of the cultures are identified in Table 1. A second mixed culture was prepared from five different isolates of Salmonella serovar I 4,[5],12:i:-, which were obtained from the Iowa State University Veterinary Diagnostic Laboratory. The isolates from the Diagnostic Laboratory were from pigs from different herds with no obvious connection, so they were considered five distinctly different isolates. A third mixed culture was prepared from five non-pathogenic Escherichia coli isolates, which have been allowed for use as surrogate bacteria in validating meat processes (USDA FSIS, 2013). All of the cultures were grown independently in trypticase soy broth (TSB) at 37oC for 18-24 hours. The respective mixed cultures (Salmonella reference, Salmonella I 4,[5],12:i:- and E. coli surrogates) were prepared by combing the individual cultures into a single mixed culture.

Inoculation: The pork meat was inoculated by immersion into the mixed cultures for 5 minutes. The inoculated meat was allowed to drain and dry at room temperature in a laminar flow hood for 10 minutes and then processed.

Intervention treatments: The inoculated pork was processed with the following intervention treatments.

a) Control, no treatment
b) washed with tap water
c) washed with hot water (74oC, 165oF)
d) water rinse followed by 2% acetic
e) water rinse followed by 2% lactic
f) hot water rinse followed by 2% acetic
g) hot water rinse followed by 2% lactic

The intervention treatments were applied by immersing the inoculated meat samples into 30 ml of water or acid in a 50 ml centrifuge tube. The inoculated meat samples were vortexed for 10 seconds, and the sample removed and immediately placed into 0.1% Buffered Peptone Water (BPW). Microbiological Analysis: The samples were homogenized and serially diluted in 0.1% BPW, and enumerated by surface plating using the one-step thin agar layer (TAL) method (Kang and Fung 2000), with Violet Red Bile Glucose agar as the selective medium and trypticase soy agar as the non-selective medium. The plates were incubated at 37oC for 24 – 48 hours, and the sample populations determined by standard methods.

Storage Studies: The non-pathogenic E. coli surrogates were not evaluated in the storage studies, as the survival of the bacteria during refrigerated storage has previously been evaluated (Marshall et al., 2005; Keeling et al., 2009). The intervention treatments which were evaluated were water (control), 3% acetic acid and 3% acetic acid at 55^{o}C (131^{o}F), following the same protocol as previously described. However, after the intervention was applied, the inoculated meat was ground through a 1/8th inch (3

mm) plate. The ground meat was transferred to sterile bags and analyzed on the day of treatment and after 14 days storage at 5°C (41°F).

Heat Resistance Studies: Heat resistance was determined using the method described by Redemann et al. (2018). Briefly, 2 grams of the ground meat was placed in a sterile bag and hand massaged to produce the thinnest possible sample. The samples were completely immersed in a water bath set at 55°C (131°F) and timing began when the samples reached 55°C . The samples were removed at appropriate time intervals and analyzed as previously described. The temperature and sampling times were selected based on the previously published results (Redemann et al, 2018). Samples were evaluated on the day of intervention treatment and after 14 days of storage at 5°C (41°F).

Experimental Design and Data Analysis: The experiments were independently replicated three times. The sample populations were converted to log10 colony forming units/ cm2 (cfu/cm2) and analyzed using SigmaStat (Systat Software; https://systatsoftware.com/products/sigmastat/).

Results:

Objective 1: The results of the intervention studies are presented in Table 2. There were differences observed between intervention ($P<0.001$) and bacteria ($P<0.001$), although the difference is due to the interaction between bacteria and intervention ($P=0.013$). When compared across all interventions, there was no difference ($P>0.8$) between the three different inocula. Compared to the Control (no treatment), all of the interventions reduced the populations of the inoculated bacteria. As expected, hot water had a greater impact when compared to room temperature (tap) water, and the combination of hot water and acetic or lactic acid resulted in the greatest microbial reductions.

Objective 2: The results of the experiments are presented in Table 3. There was an effect of treatment ($P<0.001$), but no observed differences between the two types of Salmonella serovars ($P=0.156$) or time ($P=0.169$).

Objective 3: The results of the heat resistance studies are shown in Table 4. There was no observed effect of intervention treatment on the heat resistance of the Salmonella serovars ($P=0.387$). There were observed differences between the two types of serovars, with Salmonella serovar I 4,[5],12:i:- being less resistant to heat (smaller D10 value) than the reference Salmonella serovars. There was also an effect of storage time ($P<0.001$), with the D10 values at 14 days being less than those observed on the initial day.

Discussion:

The results of Objective 1 demonstrate that Salmonella serovar I 4,[5],12:i:- is no more resistant to carcass interventions than the usual Salmonella serovars that have been documented the years. The results also show that the established non-pathogenic E. coli surrogates are also useful indicators of Salmonella serovar I 4,[5],12:i:- for carcass interventions.

The results of Objective 2 demonstrate that there is no difference in the response between the mixed reference culture of Salmonella serovars and the mixed culture of Salmonella serovar I 4,[5],12:i:- to either intervention or to storage at refrigeration temperatures.

The results of Objective 3 indicate that Salmonella serovar I 4,[5],12:i:- does not exhibit heat resistance greater than the reference strains of Salmonella, and is in fact more sensitive to heat. An important note is that the heat resistance of both serovar groups decreased after refrigerated storage for 14 days.

References:

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Table 1: Bacterial cultures used for inoculation.

Inoculum Type	Cultures
Reference *Salmonella*	*Salmonella enterica* subsp. *enterica* serovar Choleraesuis (ATCC 13312) *Salmonella enterica* subsp. *enterica* serovar Enteritidis (ATCC 4931) *Salmonella enterica* subsp. *enterica* serovar Newport (ATCC 6962) *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ATCC 700720) *Salmonella enterica* subsp. *enterica* serovar Heidelberg (ATCC 8326)
***Salmonella enterica* serovar I 4,[5],12:i:-**	ISU-SAL0233-19 ISU-SAL0234-19 ISU-SAL0235-19 ISU-SAL0236-19 ISU-SAL0237-19
***Escherichia coli* surrogates**	BAA-1427, BAA-1428, BAA-1429,BAA-1430, BAA-1431

Table 2: Bacterial populations of pork trim processed with different interventions.

Treatment	Reference *Salmonella*	*Salmonella enterica serovar* I 4,[5],12:i:-	*Escherichia coli* surrogates
Control, no treatment	7.3[a]	7.2	7.3
tap water wash	5.8	6.2	6.3
hot water (74°C, 165°F) wash	4.8	4.9	5.0
Tap water followed by 2% acetic	5.9	6.1	6.1
Tap water followed by 2% lactic	6.0	6.1	6.0
hot water followed by 2% acetic	4.9	4.8	5.0
hot water followed by 2% lactic	4.6	4.9	4.9

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

Table 3. Survival and recovery of *Salmonella* serovars from intervention treated ground pork after intervention and after storage at 5°C (41°F). The results are the difference between to populations in the control (water washed) samples and the intervention processed samples.

Treatment	Day	Reference *Salmonella*	*Salmonella enterica serovar* I 4,[5],12:i:-
3% Acetic Acid	0	0.95[a]	0.86
	14	0.84	0.73
3% Acetic Acid (55°C)	0	1.31	1.33
	14	1.35	1.20

A Difference in population = $\log_{10}$ cfu/g of population in water washed (control) samples - $\log_{10}$ cfu/g of population in intervention processed samples

Table 4. Decimal reduction values of of *Salmonella* serovars from intervention treated ground pork after intervention at 55°C and after storage at 5°C (41°F).

Treatment	Day	Reference *Salmonella*	*Salmonella enterica serovar* I 4,[5],12:i:-
Water	0	0.602[a]	0.374
	14	0.335	0.255
3% Acetic Acid	0	0.515	0.359
	14	0.343	0.281
3% Acetic Acid (55°C)	0	0.363	0.378
	14	0.313	0.300

A Decimal reduction time in minutes.