The Prevalence of Food-borne Pathogenic Organisms in Swine and Pork: A Pilot Survey and Demonstration Project from Production Farm to Dressed Carcasses

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Summary and Implications

This project was unable to demonstrate a protective effect of All in-All out (AIAO) over continuous flow production systems for swine from organisms of food safety interest at the abattoir. It provided valuable information about the ecology of *Salmonella* spp., *Campylobacter* spp. and *Yersinia enterocolitica* on farms and the abattoir. Implanted electronic microchips have been demonstrated as feasible for carcass identification from farm to abattoir cooler. Doubts about predictive values of commonly used detection procedures for on-farm prevalence of these organisms are raised. A new paradigm to explain the nexus of on-farm activities on the microbiologic status of pigs presented to the abattoir is advanced. These studies may substantially refocus farm to abattoir HACCP plans for microbial contamination.

Introduction

Implementation of the Pathogen Reduction Act and Hazard Analysis Critical Control Points (HACCP) Regulations of 1996 has changed packers and producers roles in control of microbial contamination of meat and poultry products. *Salmonella* spp., *Campylobacter* spp. and *Yersinia* spp. are pathogens of food-borne interest that are found in swine production and processing facilities. It has been proposed that AIAO production of swine reduces *Salmonella* spp. prevalence.

Project objectives were: 1) to compare AIAO and continuous flow production systems and their effects on *Salmonella* spp. prevalence on-farm and at the abattoir; 2) to supplement the scant epidemiologic information about *Y. enterocolitica* and *Campylobacter* spp. in commercial swine operations in two geographical areas of the USA; 3) to compare on-farm fecal microbial results with carcass and intestinal isolation rates for *Salmonella* spp., *Campylobacter* spp. and *Y. enterocolitica;* and 4) to gain experience with an implanted electronic identification system from ante mortem to the cooler.

Materials and Methods

Four commercial production units, two each AIAO and continuous flow management, were selected in Iowa and North Carolina, respectively (total 8 farms). Two cohorts were followed sequentially on each production unit. Criteria for selection included the presence of management, biosecurity and genetics typical for their respective production type. A common slaughter facility was identified in each state to reduce in-plant variability for microbial controls.

The study design was a cohort format with 60 randomly selected nursery-aged pigs per cohort identified on each farm to assess microbial prevalence within the production unit. This sample size provides a 95% statistical probability of detecting a 5% infection rate of the organisms in question. The organisms of interest were *Salmonella* spp., *Campylobacter* spp., and *Y. enterocolitica*. Each Iowa pig was individually identified behind the right rear dew claw with an electronic implant supplied by the AVID Company. The North Carolina pigs were individually identified by eartag.

Each group was sampled within three weeks of weaning and then at eight week intervals until market weight. Within 48 hours of market a final sampling occurred. At each sampling period a 1 gm fecal loop samples was collected from each animal for *Salmonella* and *Campylobacter* using standard protocols (1,2). Tonsil scrapings were taken for isolation of *Y. enterocolitica* by the methods of Dr. Irene Wesley (2). *Yersinia* and *Campylobacter* culture positives were further characterized using multiplex PCR assays (3). Blood samples were obtained from each pig for serologic evaluation using the Danish mixed-ELISA (4). At pre-slaughter sampling each animal was slap tattooed with a unique number for carcass

identification during the harvest process.

At the abattoir the carcasses were swabbed using the standard USDA-FSIS microbial detection protocol. Swabs were cultured for *Salmonella* spp., *Campylobacter* spp., and *Y. enterocolitica* using standardized laboratory procedures (1,2,3). Each Iowa carcass was examined for readability of the electronic implant and a muscle sample (crus of diaphragm) was taken for the mixed ELISA. The head from each carcass was harvested, marked with the slaughter order and tonsil removed. The intestines were identified to maintain identity of viscera into the large intestine harvesting area. Ileo-cecal lymph nodes, colon or cecum tissues, and tonsils were placed in separately identified whirl pak bags and transported on ice to the laboratory.

The swine transport vehicle was sampled prior to loading and after unloading at the packing plant. These samples were cultured for *Salmonella* spp. to detect shedding during transit. Iowa transit times varied from 30 to 150 minutes depending on the distance from the packing plant. The North Carolina groups had transit times from 60 to 240 minutes. Iowa pigs were allowed to rest for at least two, but not more than four, hours before slaughter. To comply with plant requirements in North Carolina, pigs were held in lairage overnight and slaughtered as the first group in the morning.

Results and Discussions

Fecal *Salmonella* spp. cultures at the farm level in the nursery, grower and finish stages were limited in both Iowa groups (Table 1). At pre-slaughter sampling one farm presented a 12% isolation rate for *S. typhimurium* in each sample period. All other periods were negative with the exception of one nursery animal that tested positive for *S. worthington* (a serotype regularly isolated in previous studies from the breeding herd of this unit).

In contrast, positive cultures were obtained from 17-49% of ileo-cecal lymph nodes, cecal contents or colon (Table 2). These isolates demonstrated substantial diversity and prevalence when compared with on-farm attempts. Up to 10 different serotypes were observed within a cohort. The same serotypes from ileo-cecal lymph nodes, colon or cecal contents was not consistently observed in individual animals, and dominant serotypes were different for each cohort. Distribution differences were not attributable to the production system. Carcass swabs were negative for all in each cohort. The mixed ELISA was negative for almost all cohorts with the few positives detected in finish or pre-market periods.

The North Carolina herds presented a more variable on-farm isolation pattern. *S. typhimurium* and *S. typhimurium* var. *copenhagen* were the most common serotypes. When present these serotypes recurred in subsequent sample periods. When non-host adapted species were found they did not recur with the same frequency.

In contrast to the Iowa herds, North Carolina cohorts demonstrated more consistency in isolates from ileo-cecal lymph nodes when compared with cecal cultures. Many of these isolates were *S. typhimurium* or *S. typhimurium* var. *copenhagen*. Cecal cultures for each cohort contained these seroptyes, if found in ileo-cecal nodes, with additional non-adapted serotypes. As with the Iowa cohorts none of the carcass swabs were positive for *Salmonella* spp.

The mixed ELISA results from the North Carolina cohorts were not consistent with the on-farm isolation results. At the pre-market period all previous positive pigs in the first cohort set had converted to negative status. In the second cohort set a large number of serologic positives could be identified at the nursery and finishing phase but not in the growers of three of four cohorts. These inconsistencies could not be explained.

Campylobacter spp. fecal isolation from all stages of production exceeded 80%. The in-plant lymph node homogenate isolations were more variable, ranging from 22-87%. This variation was consistent with the isolation rates from Iowa cohorts (35-88%). The first cohort set had a consistently higher isolation rate (70-88%) when compared to the second set (35-66%). Three of four Iowa cohorts in the second set had no carcass isolations while one presented 56/57 positives. The first Iowa iteration and both North Carolina sets had low carcass isolation rates. Two AIAO North Carolina cohorts were carcass negative.

Yersinia enterocolitica isolates were limited in number. Few isolates were found by tonsil scrapings or from tonsils harvested at slaughter. Ileo-cecal lymph node homogenates generated more variable results, but were not consistent with tonsil isolations. Explanations for this dichotomy are not apparent, unless antemortem environmental contamination at the plants is more sensitively detected in lymph nodes.

The *Salmonella* spp. isolation rates for all cohorts generated confusion. Consistency of *Salmonella* spp. isolation from these selected sites in each carcass was lacking. It was not uncommon to find a positive ileo-cecal lymph node and a negative colon or cecal content in the same animal, or vice versa, resulting in aggregate contamination rates of greater than 50% in all sampled groups. Such rates were not predicted by the found on-farm isolation results. Explanations for this observation lead to the positing of several scenarios that may significantly impact on-farm HACCP implementation strategies:

1. The isolations in the abattoir are reflective of the true production site status and the ante mortem tests used in this project (fecal loops and mixed ELISA) performed so poorly as to be non-predictive at the farm level.
2. The stress of movement and lairage encouraged multiplication in gut-associated tissues to levels sufficient to be detected.
3. The gut-associated tissues were contaminated during transport and/or lairage after the animals left the production site.

The data collected in this project can not provide conclusive support for any of these hypotheses because of the study design. Obviously the correct answers to these observations are central to formulating farm and processor strategies to reduce *Salmonella* spp. contamination of pork products. This question has spurred additional field and laboratory research (McKean, Hurd, Griffith, Rostagno) to determine the more likely source of the elevated gut-

associated tissue isolation rates observed. Preliminary data by this team would indicate that the third scenario – contamination during transport and lairage at the abattoir – is the more likely causation of these findings. This observation may provide a substantial platform from which to develop farm to cooler HACCP plans with a decidedly different focus from current efforts focused solely at the farm level.

Campylobacter spp. isolates in this study were common and almost uniformly determined to be *C. coli.* In both the North Carolina and Iowa cohorts the prevalence of *Campylobacter* spp. ranged from 80-100% in each sampling period. Numerically the nursery was the lowest period, but in each case at the next sampling the prevalence was approaching 90-100%. This demonstrates that the fecal prevalence for *Campylobacter* spp. in swine is high under a range of production practices. All but one isolate of these on-farm samplings was *C. coli*, generally regarded as a relatively innocuous organism from a food safety perspective. The other isolate was *C. jejuni* that was found in a finishing animal. The high fecal prevalence in all stages of grow-finish limits the likelihood under current management practices of on-farm interventions that could reduce *Campylobacter* spp. presentations to the abattoir in live swine.

The fourth objective, limited to the Iowa cohorts, was to gain experience with electronic microchips and their retention through the harvesting process. The chips were inserted in an approved site – under the dew claw in the right hind leg. This site proved easy and convenient to access in nursery aged pigs. Retention of the microchips was acceptable in all groups. Less than1% of the total pigs lost in this study could not be accounted for from death loss.

This technology enables the efficient identification of carcasses back to the farm of origin, and offers opportunities to segment markets by guaranteed attributes at the consumer level while using the efficiencies of the modern high capacity facilities for harvesting activities.

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Table 1. Iowa *Salmonella* spp. isolations from farm periods.

	Nursery	Grower	Finish	Pre-Market
Round 1				
Farm 1 (CF)	0/60	0/59	0/58	0/60
Farm 2 (CF)	0/60	0/45	0/39	0/38
Farm 3 (AIAO)	0/60	0/55	0/51	6/51
Farm 4 (AIAO)	0/60	0/52	0/51	0/53
Round 2				
Farm 1 (CF)	0/60	0/60	0/60	0/60
Farm 2 (CF)	0/51	0/50	0/47	0/46
Farm 3 (AIAO)	1/60	0/57	NA	6/50
Farm 4 (AIAO)	0/60	0/60	0/59	0.59

Table 2. Iowa *Salmonella* spp. isolations from plant tissues.

	Carcass Swabs	Cecum	Colon	Ileo-cecal Lymph Nodes
Round 1				
Farm 1 (CF)	0/59	NA	5/22	4/22
Farm 2 (CF)	0/34	2/18	6/29	7/29
Farm 3 (AIAO)	0/50	9/49	15/49	9/49
Farm 4 (AIAO)	0/51	13/45	14/45	7/45
Round 2				
Farm 1 (CF)	0/60	31/60	NA	20/60
Farm 2 (CF)	0/41	21/35	NA	10/35
Farm 3 (AIAO)	0/50	13/47	NA	32/47
Farm 4 (AIAO)	0/59	23/41	NA	8/41

Table 3. Iowa Salmonella mixed ELISA serology results.

	Nursery	Grower	Finish	Pre-Market	Diaphragm
Round 1					
Farm 1 (CF)	0/60	0/56	1/57	2/59	3/18
Farm 2 (CF)	0/60	0/45	0/39	2/38	0/10
Farm 3 (AIAO)	0/60	0/55	0/51	0/45	0/48
Farm 4 (AIAO)	0/60	0/52	0/47	0/53	0/51
Round 2					
Farm 1 (CF)	0/60	0/60	0/59	2/44	0/36
Farm 2 (CF)	0/51	0/50	0/47	1/46	0/38
Farm 3 (AIAO)	0/60	3/57	NA	0/50	0/43
Farm 4 (AIAO)	0/60	0/60	0/59	1/59	1/58

Table 4. North Carolina *Salmonella* spp. isolations from farm periods

	Nursery	Grower	Finish	Pre-Market
Round 1				
Farm 1 (AIAO)	1/60	6/59	0/55	9/31
Farm 2 (CF)	0/60	0/59	0/57	0/59
Farm 3 (CF)	7/60	0/57	20/53	1/49
Farm 4 (AIAO)	6/60	3/59	1/54	1/55
Round 2				
Farm 1 (AIAO)	18/60	24/60	13/60	21/47
Farm 2 (CF)	0/60	3/60	0/59	0/59
Farm 3 (CF)	2/60	3/60	0/60	1/55
Farm 4 (AIAO)	1/60	5/60	6/55	18/51

Table 5. North Carolina *Salmonella* spp. isolations from plant tissues.

	Carcass Swabs	Cecum	Colon	Ileo-cecal Lymph Nodes
Round 1				
Farm 1 (AIAO)	0/31	16/31	NA	11/31
Farm 2 (CF)	0/50	21/59	NA	8/59
Farm 3 (CF)	0/49	21/49	NA	5/49
Farm 4 (AIAO)	0/53	5/53	NA	3/53
Round 2				
Farm 1 (AIAO)	0/47	36/47	NA	24/47
Farm 2 (CF)	0/59	1/59	NA	0/50
Farm 3 (CF)	0/55	36/55	NA	7/55
Farm 4 (AIAO)	0/51	21/51	NA	27/51

Table 6. North Carolina Salmonella mixed ELISA serology results.

	Nursery	Grower	Finish	Pre-Market	Diaphragm
Round 1					
Farm 1 (AIAO)	0/60	23/60	11/55	0/31	NA
Farm 2 (CF)	0/60	0/59	0/59	0/59	NA
Farm 3 (CF)	0/60	0/57	6/54	0/49	NA
Farm 4 (AIAO)	0/60	1/59	1/57	0/55	NA
Round 2					
Farm 1 (AIAO)	0/60	0/55	0/59	NA	NA
Farm 2 (CF)	0/59	0/56	19/49	NA	NA
Farm 3 (CF)	15/46	0/54	8/58	NA	NA
Farm 4 (AIAO)	0/60	4/54	4/53	NA	NA