RESEARCH REPORT

PORK SAFETY

Title: Strain Specific Typing Bacterial Pathogens in the Pork Industry Chain
NPB #99-026

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I. Abstract

The incidence of *Listeria monocytogenes*, *Salmonella* spp. and *Yersinia enterocolitica* was assessed at one small and one medium-sized slaughterhouse and five farms supplying hogs to these slaughterhouses. Four hogs from each farm were randomly selected and sampled by swabbing before stunning, after scalding/singeing, after evisceration, after final carcass washing and after chilling. Environmental samples were collected from receiving (truck, holding pens), processing (dehairing machine, band saw, drains) and storage areas (cooler walls). Five composite environmental samples of manure, feed, trough water and pen areas were collected from farms supplying hogs to the slaughterhouses. Samples were examined for *L. monocytogenes, Salmonella and Y. enterocolitica* using USDA/VIDAS, FDA/VIDAS, and the FDA methods, respectively. *L. monocytogenes* was present in 28 of 565 (5.0%) samples, 17 (60.7%) of which were environmental. Six samples from trucks off-loading hogs for slaughter and four samples from the dehairing machine yielded *L. monocytogenes. L. monocytogenes* was recovered from one carcass swab after evisceration in the post-slaughter processing area. *Salmonella* was present in 24 of 509 (4.7%) samples, 12 of which (50%) were environmental, including 4 samples from trucks. Two samples from the dehairing machine yielded *Salmonella*, whereas 4 and 2 samples tested positive for *Salmonella* at evisceration and prechill/final wash, respectively. However, neither *L. monocytogenes* nor *Salmonella* were isolated from carcasses after 16-18 h of chilling. Several floor drain samples from the slaughter and meat-processing/packing areas were positive for *L. monocytogenes* and *Salmonella*. Thirty-one isolates of *S.* Typhimurium were serotyped as O-5 negative. No *Yersinia spp*. was identified from samples collected at the small slaughterhouse after which no further analyses were conducted. PFGE analysis yielded 9 different clusters of *L. monocytogenes*. Three isolates yielded PFGE patterns that were similar to a previously determined clinical pattern from a foodborne outbreak. Eight isolates of *S.* Typhimurium DT 104 were presumptively identified using PFGE.

II. Introduction

L. monocytogenes, *Salmonella* spp. and *Y. enterocolitica* are foodborne pathogens of major concern in pork production. According to the Center for Disease Control and Prevention (CDC), *Salmonella* accounted for 357 outbreaks from 1993-1997 which involved 32,610 cases and 13 deaths. CDC also reported that *S.* Typhimurium is the most frequently isolated serotype, with many isolates identified as *S.* Typhimurium DT 104 – an emerging multi-antibiotic resistant strain. *L. monocytogenes* caused 3 major outbreaks that included 100 cases and 2 deaths during the same period, where only 2 (27 cases and 1 death) were traced to *Y. enterocolitica.* Pork was the vehicle of transmission in 14 outbreaks involving

638 cases and 1 death. Fecal shedding and growth of pathogens in the farm environment can lead to subsequent contamination of the slaughterhouse environment and finished product. Most outbreaks are associated with intensively reared weaned pigs. Infected animals and contaminated feed are supposedly the primary routes for introducing these pathogens into herds.

III. Objectives

The objectives of this study were to: (a) identify the prevalence of *L. monocytogenes*, *Salmonella* spp. and *Y. enterocolitica* on hogs and in two slaughterhouses before, during and after processing and (b) use Pulsed-Field Gel Electrophoresis (PFGE) to track the transmission of *L. monocytogenes, Salmonella* spp. and *Y. enterocolitica* in the pork production chain.

IV. Procedures

Sampling. Six site visits were made to one small- (~200 head/week) and one medium-sized (>1000 heads/week) commercial slaughterhouse during 4 to 6 months. During visits, four hogs from each of two farms supplying the small-sized slaughterhouse and four hogs from each of three farms supplying the medium-sized slaughterhouse were randomly selected and followed through processing (Figure 1). Using separate sterile sponges rehydrated in sterile Butterfield's buffer solution, the ham, belly and jowls of these carcasses were sampled. (a) before stunning, (b) after scalding/singeing/skinning, (c) after evisceration, (d) after final carcass washing/rinsing, and (e) after chilling, fifteen. environmental samples (Table 1) also were collected from receiving (e.g. truck, holding pen), processing (e.g. equipment, processing, floors, drains) and storage areas (e.g. cooler walls, floors, drains). To trace the contamination back to the farm level, farms that supplied the slaughterhouses with hogs were visited. During on-farm visits, five composite environmental samples were collected with primary emphasis given to manure, feed, trough water and pen areas (Table 2).

Microbiological analysi.s. *L. monocytogenes* was isolated using the USDA (4) and VIDAS (bioMerieux, Inc, Hazelwood, MO) procedures (3). Samples were enriched in Fraser broth containing ferric ammonium citrate, followed by Fraser broth without ferric ammonium citrate. Environmental samples were separately enriched using UVM medium. Fraser broth enrichments without Ferric Ammonium Citrate and UVM broth were screened for *L. monocytogenes* using the VIDAS automated ELISA assay system. These enrichments were also streaked to Modified Oxford Agar. Suspected colonies were purified and confirmed biochemically and/or serologically. *Salmonella* was detected using the FDA BAM (1) and VIDAS procedures. Recovery of *Salmonella* involved pre-enrichment in lactose broth followed by enrichment in both selenite cystine and tetrathionate broths. After additional enrichment in M-broth, cultures were screened for *Salmonella* using the VIDAS automated system (bioMerieux, St.Louis, MO). M-broth cultures were surface-plated on Hektoen enteric, xylose lysine desoxycholate and bismuth sulfite agars for isolation using the FDA cultural method. Suspected *Salmonella* colonies were purified and biochemically confirmed using API 20E strips (bioMerieux, St.Louis, MO), followed by serological identification. *Y. enterocolitica* was recovered according to the FDA BAM method (5) using cold enrichment in peptone sorbitol bile broth followed by plating on MacConkey agar. Suspect *Yersinia* colonies were streaked to Yersinia Selective agar and biochemically confirmed using API 20E test strips (bioMerieux, Inc, Hazelwood, MO).

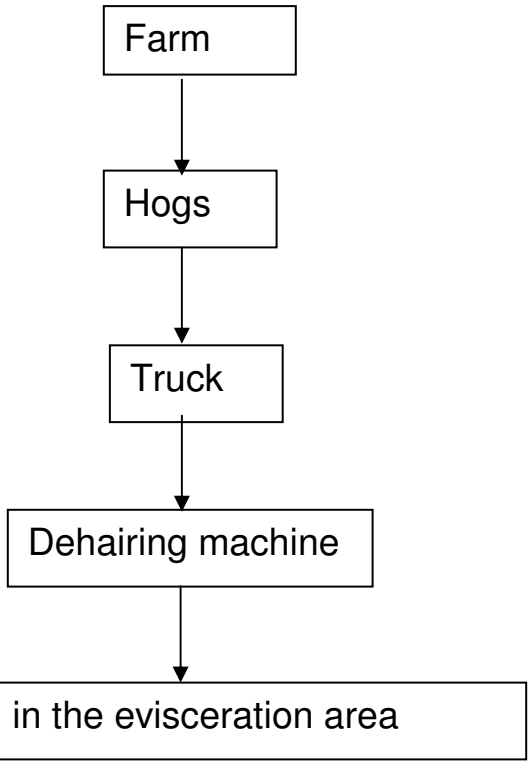
PFGE analysis. Agarose-embedded DNA was prepared following standard PulseNet procedures (2). DNA from *L. monocytogenes* was digested separately using AscI (NewEngland Biolabs, Beverly, MA) and ApaI (Promega, Madison, WI). *Salmonella* DNA was digested using XbaI (Roche, Indianapolis, IN). Electrophoresis was performed using a CHEF DRII system (BioRad, Hercules, CA) with 1% Seakem Gold agarose, a running length of 18 hours, and switch times of 4-40 seconds for *L. monocytogenes* and 2.2 – 63.8 seconds for *Salmonella*. Gel images were acquired with GelDoc 2000

software (BioRad, Hercules, CA) and analyzed using Molecular Analyst Fingerprinting software (BioRad, Hercules, CA). The clusters patterns used were those available from the database of the Molecular Biology section of the Michigan State Reference Lab under PulseNet. Isolates were considered to be indistinguishable if no pattern differences were identified, probably related if their patterns differed by 1-2 bands or one genetic event, possibly related if their patterns differed by 3 bands or 2 genetic events, and unrelated if their patterns differed by more than 3 bands or more than 2 genetic events

V. Results

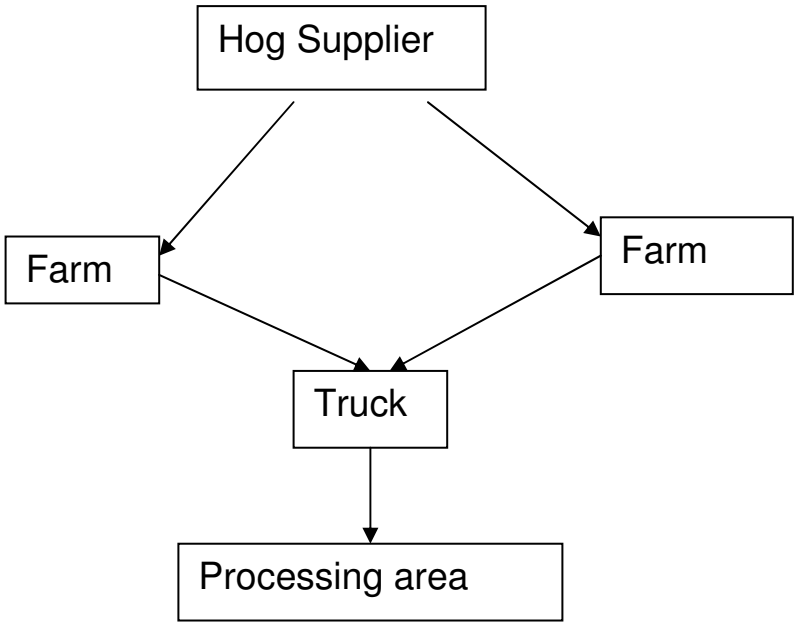
Microbiological analysis: Overall, 28 of 565 (5.0%) samples were positive for *L. monocytogenes,* most of which were environmental. Six samples from trucks delivering hogs to slaughterhouses yielded positive results. Samples of hair collected behind the dehairing machine and drain samples from the evisceration section of the plant were positive on multiple site visits. One tongue was presumptively positive for *L. monocytogenes*. Twenty-four of 509 (4.7%) samples yielded *Salmonella* spp. Although 50% of the positive samples were environmental, several samples at evisceration and prechill/final wash also were positive. Thirty isolates from the small slaughterhouse and the farms were *S*. Typhimurium, 22 of which were serotyped as O-5 negative. Different *Salmonella* strains were detected at the medium slaughterhouse and its corresponding farms. *S.* Derby and *S.* Newport were found in 4 (3 prechill & 1 alley) and 2 (2 evisceration) samples, respectively. One isolate of *S.* Typhimurium also was found in hairs behind the dehairing machine at the medium slaughterhouse. *Yersinia* was not detected in any samples collected from the small slaughterhouse or related farms. Consequently, no further samples were examined for *Yersinia spp*.

PFGE analysis*:* Nine different clusters of *L. monocytogenes* were identified using PFGE (Table 4). Three isolates were similar to a previously determined clinical PFGE pattern that was responsible for an outbreak of foodborne listeriosis. Isolates belonging to PFGE type 1 were present in six samples. PFGE type 2 was present in many areas of the slaughterhouse (i.e., truck, behind the dehairing machine, drain) and was also found on farm S suggesting the following transmission route:



Thirty-one isolates of *S.* Typhimurium were biochemically confirmed

characterized using PFGE. Based on PFGE analysis, 8 isolates of *S*. Typhimurium DT 104 were identified from the small plant. *S.* Typhimurium was found on one of the farms and in the small slaughterhouse. Since samples from the other farm also yielded similar isolates, *Salmonella* may have been transferred onto the farm from a different source and entered the slaughterhouse through in-coming hogs as follows:



For both *L. monocytogenes* and *Salmonella* spp., transmission from the outside environment to the farm and through trucks to processing areas is suggested. Similar PFGE patterns in different isolates from different farms suggest possible entry of the organism into the herd through a common hog supplier.

VI. Summary

- *L. monocytogenes* was found in 28 of 565 (5.0%) samples (17 slaughterhouse environmental areas, 5 from hogs' back and carcasses, 6 farm environmental sites). After 16 to 18h of chilling, none of the carcasses yielded *L. monocytogenes*.
- PFGE yielded 9 different clusters of *L. monocytogenes*, of which PFGE types 1 and 2 were present in 6 and 9 samples, respectively.
- PFGE identified the presence of types 1 and 2 in samples collected during six visits for over 6 months.
- *Salmonella* spp. were found in 24 (4.7%) of 509 samples, (12 of which slaughterhouse environmental areas, 9 hogs' backs and carcasses, and 3 farm environmental sites.)
- Of the total 38 isolates, 31 were *Salmonella* Typhimurium O-5 negative strains, 4 were *S.* Derby, 2 were *S.* Newport and 1 was *S.* Agona. All non-Typhimurium strains were isolated from carcass swabs.
- Eight isolates were from the small slaughterhouse presumptively identified as *S.* Typhimurium DT 104 using PFGE.
- *Yersinia enterocolitica* was not found in any samples during the first 6 months visits.

VII. References

1. Andrew, WH, Hammack, TS, and Amaguana, RM. 1998. *Salmonella*, in FDA Bacteriological Manual, 8th ed., AOAC, Gaithersburg, MD.
2. Centers for Disease Control and Prevention. 2000. Standardized Molecular Subtyping of Foodborne Bacterial Pathogens by Pulsed-field Gel Electrophoresis. US DHHD, Atlanta, GA.
3. Hitchins, A. 1998. *Listeria monocytogenes*, in FDA Bacteriological Manual, 8th ed., AOAC, Gaithersburg, MD.
4. McClain, D., and Lee, W. 1994. FSIS Method for the Isolation and Identification of *Listeria monocytogenes* from Processed Meat and Poultry Products. USDA, FSIS Microbiology Division, Beltsville, MD.
5. Weagant, SD, Feng, P, and Stanfield, JT. 1998. In *Yersinia enterocolitica and Yersinia pseudotuberculosis,* FDA Bacteriological Manual, 8th ed., AOAC, Gaithersburg, MD.

VIII. Abstract

1. Sharma, R.K., E.T. Ryser, and W.N. Osburn. 2000. Prevalence of *Listeria monocytogenes, Salmonella Typhimurium* and *Yersinia enterocolitica* on incoming hogs and fresh pork during and after slaughter. Proc. Ann. Mtg. Intern. Assoc. Food Prot., Atlanta, GA,, Aug. 6-9, Abst. P098.

Table 1. Environmental samples from slaughterhouses during each visit

Site	Sample	Method & quantity
Day I : Pre-slaughter		
Truck[a]	Fecal mat., floor	Scoop, 50g
Alley	Fecal mat., floor	Scoop, 50g
Chillroom wall	Swab from the chillroom wall	Swab
Scalding water	Water from the scalding tank	25 – 50ml of water
At the dehairing machine	Hairs scooped out from behind the dehairing machine	25g, hair scoop
Drain	central drain near evisceration	Swab
Day II : Pre-fabrication		
Band saw	Band saw	Swab
Breaking table	Breaking table	Swab
Loin table	Loin table	Swab
Shoulder-trim table	Shoulder-trim table	Swab
Belly table	Belly table	Swab
Ham table	Ham table	Swab
Conveyor belt	Conveyor belt	Swab
Drain in the processing room	Drain in the processing room	Swab

[a] Two samples taken for each of the two farms.

Table 2. Farm Samples

Site	Sample	Method and quantity
Pen floor/Alley	Composite	Scoop, 50g
Feed	Composite	Scoop, 50g
Water pipe nozzle or water trough	Composite	Swab from the nozzle tip
hogs' back	A single composite. Sampled from 4 hogs from each farm.	Swab, with neutralizing buffer solution
Fecal matter	Composite	Scoop, 50g

Figure 1. Pork processing

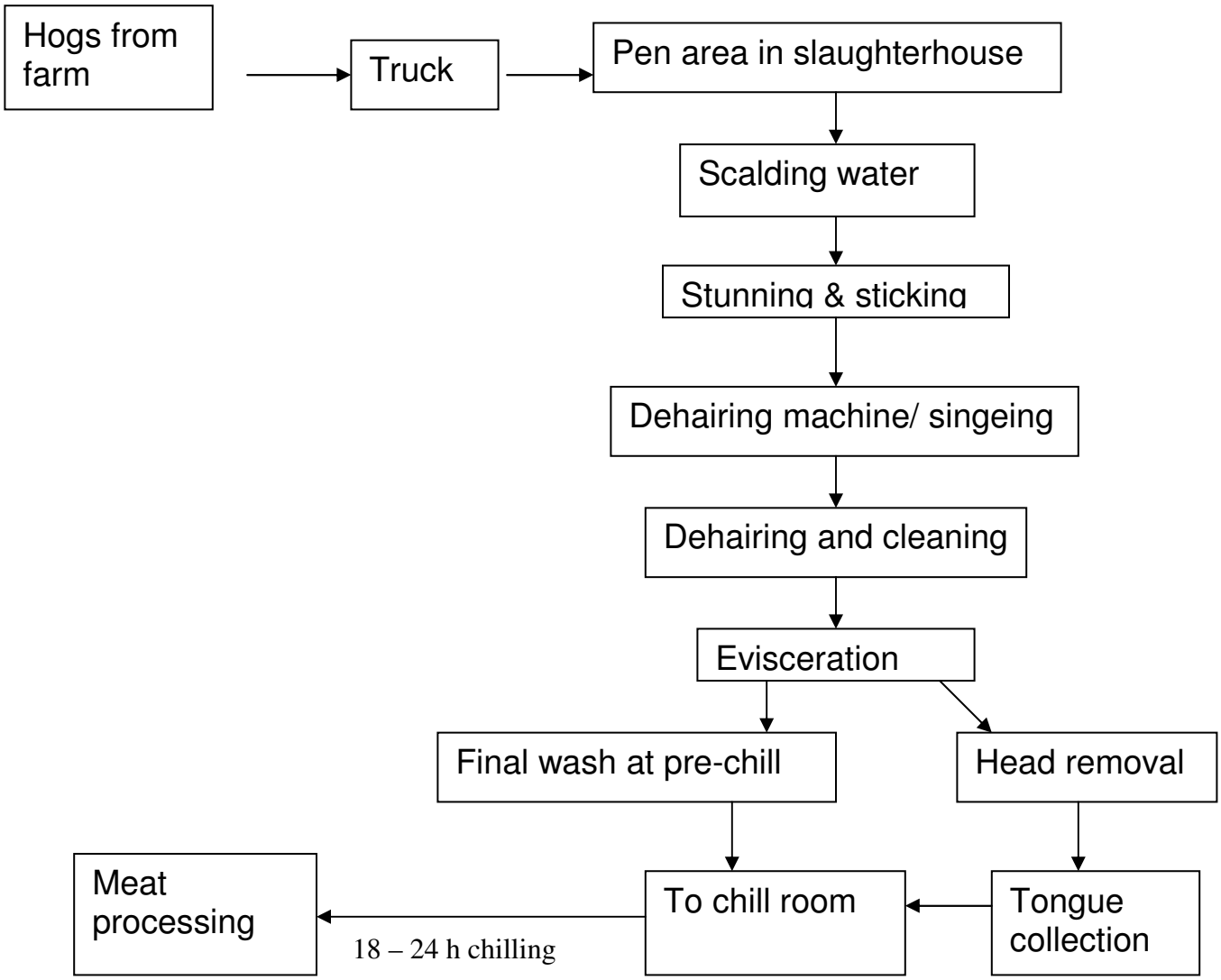


Table 3. Incidence of *L. monocytogenes* and *Salmonella* spp. in the pork production chain

Sample	Location	V.	**L. monocytogenes**	*Salmonella* spp.	Number of samples
Hogs and carcasses	Hogs' back		3	2	19
	Carcass at dehairing (I)			1	72
	Carcass at evisceration (II)		1	4	72
	Carcass at prechill (III)			2	72
	Tongues		1	Not processed	56
	Carcasses after chilling				72
	Total		5 (1.7%)	9 (3%)	363
Environmental samples	Truck		6	4	11
	Alley		2	1	13
	Hairs		4	2	12
	Drain at evisceration		2	2	12
	Saw				7
	Breaking table		1		12
	Loin table				12
	Shoulder-trim table				12
	Belly-ham / belly table				12
	Ham table				3
	Conveyor belt				3
	Drain in Processing		2	3	13
	Total		17 (13.3%)	12 (9.4%)	122
Farm	Farm Hogs		2	1	16
	Farm Feed		1		16
	Farm Alley		3	2	16
	Water nozzle				16
	Fecal matter				16
	Total		6 (7.5%)	3 (3.75%)	80
	TOTAL		28 (5.0%)	24 (4.7%)*	565

* - 4.7% of 509 samples processed for *Salmonella* spp.

Table 4. *L. monocytogenes* isolated from slaughterhouses and delivering farms

PFGE Pattern	Trip	Slaughterhouse Day 1	Slaughterhouse Day II	Farm S	Farm D
1	I	Truck S			
	II	Truck S		Hogs' back	
	III	Truck D		Farm alley	
	VI	Hogs' back D			
2	I	Hogs' back S, Truck D	Drain in processing area	Hogs' back	
	II	Truck D, Hairs, Drain at evisceration			
	V	Hairs			
	VI		Breaking table		
3	V	Hogs' back D			
4	II	Drain at evisceration			
5	III	Hairs			
	V	Alley			
6	IV	Truck S			Farm alley
7	V	Hairs			
8	VI	Truck S			
9	I	Truck D			

Table 5. *Salmonella* spp. isolated from slaughterhouses and delivering farms

Strain	PFGE Pattern	Trip	Plant Day I	Plant Day II	Farm S	Farm D
O – 5 neg.	1	I	Hairs, Carcass D-I, Truck D	Drain in processing area		
		II	Hogs' back D			Hogs' back
		III	Truck D, Truck S			
		IV	Carcass D-II, Hogs' back D		Farm alley	Farm alley
		V	Truck S, Drain at evisceration			
		IX	Hairs			
DT 104	2	II		Drain in processing area		
		III	Alley, Truck D			
		V	Drain at evisceration			
		VI		Drain in processing area		
Derby		VII	Carcass TO-II, Carcass TO-III			
Newport		IX	Carcass W-II			
Agona		IX	Drain at evisceration			