The Prevalence of *L. monocytogenes* in Cull Sows

I.V. Wesley[1], S. Larsen[2], S, HS Hurd[1], J McKean[2], T. Frana[2], M. Cox[3], M.G Johnson[3], R. Nannapaneni[3], A. Pinkerton-Hogness[1], and F. Rivera[1]
[1]USDA-ARS-National Animal Disease Center, Ames, IA,
[2]College of Veterinary Medicine, Iowa State University, Ames, IA.
[3]Food Science Department, University of Arkansas, Fayetteville

ASL-R1809

Summary and Implications

The goal of this study was to determine the distribution of *Listeria monocytogenes* in cull sows and their pork. Two trials were conducted at a single packing plant in 2001 (n=179 cull sows) and in 2002 (n= 160 cull sows). Fecal samples collected antemortem (trial 1) as well as animal tissues, carcass, and environmental swabs, and meat block samples collected at the abattoir (trials 1 and 2) were analyzed. When results from both trials were combined, overall L. monocytogenes was detected in five or 0.17% of the total samples (n=2,858). Specifically, *L. monocytogene*s was confirmed in a tonsil sample (0.55% of tonsils positive) and in a carcass swab sample (0.56% of carcasses) before the organic acid rinse. *L. monocytogenes* was recovered in three (1.21%) meat block samples (n=213). These data indicate that *L. monocytogenes* is present in the cull sow and their pork.

Introduction

L. monocytogenes colonizes the intestine of healthy market weight hogs and may subsequently contaminate carcasses and the abattoir environment during slaughter. In Europe, carriage rates in live hogs of up to 47% for feces and up to 60% of tonsils have been reported (8). This indicates that swine may be important reservoirs of *L. monocytogenes*.

In an earlier study, we did not recover *L. monocytogenes* from feces (0%), but cultured it from 4% of carcasses. *L. monocytogenes* was also cultured from 22% of ground pork manufactured that day from unrelated hogs slaughtered the previous day.5 A similar increase in contamination from live animals to ground meat has been reported earlier (8). The USDA Food Safety Inspection Service (FSIS) nationwide microbial baseline survey of hogs reported *L. monocytogenes* on 7.4% of hog carcasses (7). Yet in a nationwide survey in six metropolitan cities, *L. monocytogenes* was found in 27% of retail purchased ground pork (2).

One of the largest listeriosis outbreaks occurred in France and was traced to pickled pork tongue and involved 279 human cases (33% pregnancy related). A second outbreak in France of 38 patients (82% pregnancy related) incriminated contaminated pork rillettes (pate), which are served as appetizers and may be derived from cull sows. Healthy carrier hogs were thought to have introduced L. monocytogenes into the plant involved in that outbreak (3,4).

The reduction of foodborne pathogens, such as *L. monocytogenes* spp., from the farm to table has focused on market weight swine (~275 lbs), which represent ~ 95% of the pork slaughtered each year. Whereas primal cuts are derived mainly from these younger hogs, nearly 100% of whole hog sausage meat, including pepperoni and specialty sausages, is derived from cull sows (~400 lbs). The cull sow represents ~4% of the total pork market compared with market swine. Our goal was to determine the prevalence of *L. monocytogenes* in cull sows and meat blocks derived from these animals.

Materials and Methods

Cull sows Two trials were conducted. The first consisted of five sampling periods (n~40 hogs per period) from January to March 2001. Sows from a single premise were transferred to a collection point and held for 16 to 20 hours in pens bedded with fresh wood shavings. Before loading, fecal samples (10 g) were obtained by digital extraction from each selected sow using sterile gloves and deposited in sterile whirl-pak bags. Sows were transported 720 km (~10 hours) to the abattoir, held in pens for 6 to 8 hours, and slaughtered as the first animals for the day. Samples collected at slaughter included ileocecal lymph node (5-10 g), cecal contents (20 g), transverse colon contents (15 g), ventral thoracic lymph node (1-5 g), subiliac lymph node (1-5 g), sponge swabs of the left and right carcass section utilizing the standard three-site USDA-FSIS procedure (300 cm^2) before and after the organic acid rinse, and meat block (~4-inch cubes) samples.

The second trial consisted of four sampling periods (n=40 sows each) from February to April 2002. Sows were brought to a buying station and were selected based on condition scores and weight limits set by the purchasing sausage company and transported to the abattoir. In this second trial, on-farm fecal samples were not collected. Samples collected at the abattoir included ileocecal lymph node (5-10 g), cecal contents (20 g), transverse colon contents (15 g), subiliac lymph node (1-5 g), precarcass wash sponge swabs of the left and right carcass section utilizing the standard three-site USDA-FSIS procedure (300 cm^2), and meat block samples (~4-inch cubes) derived from these animals. In both trials, all samples were placed in Whirl-pak bags (Nasco, Ft.

Atkinson, WI) and transported on ice to the National Animal Disease Laboratory, Ames, Iowa.

Microbiological testing Buffered peptone water (90 ml; Remel, Lenexa, KS) was added to samples (~10 g) of feces, colon, and cecal contents and homogenized (1 minute at 260 rpm; Seward, London, UK). A 1-ml aliquot of each homogenate was added to University of Vermont (UVM) enrichment (9 ml), as described below. The ventral thoracic and subiliac lymph nodes from each carcass were pooled, placed in a sterile bag, and macerated with a rubber mallet. The ileocecal lymph nodes were processed separately. Peptone water (10 ml) was added to the macerated lymph node samples and homogenized (1 minute at 260 rpm; Seward). Aliquots (1 ml) of each tissue homogenates was added to the UVM I (9-ml). Carcass sponges were placed into UVM I (9-ml) enrichment. Primary UVM I enrichments were incubated (48 hours at 30°C), transferred to UVM II (24 hours, 30°C), and plated to PALCAM agar (48 hours, 30°C, in a microaerobic environment), as described previously (5). At least two colonies exhibiting typical *L. monocytogenes* appearance on PALCAM plate (black colonies on orange/red agar background) were subcultured to TSA slants. In trial 1, presumptive isolates were identified by a 5' nuclease (TaqMan) assay targeting the *hlyA* gene, as described (6). In trial 2, the multiplex PCR assay was used to differentiate *L. monocytogenes* from other Listeria species (9). Presumptive *L. monocytogenes* strains were then confirmed by conventional biochemical assays, serogrouped, and evaluated for virulence and antimicrobial susceptibility.

Virulence testing L. monocytogenes isolates were virulence tested using Ped-2E9 hybridoma cell lines (5 x 105/ml) at a ratio of 1,000 *L. monocytogenes* cells per each target hybridoma cells (1). Death of hybridoma cells was scored at 6 hours. Microcolonies of *L. monocytogenes* serotypes a and 4b were used as positive controls. *L. innocua* was used as a negative control. All tests were repeated in triplicate.

Antibiotic susceptibility testing Minimal inhibitory concentrations (MICs) were determined using a microdilution procedure (SensititreÆ, Sensititre Ltd.) as stated in the National Committee for Clinical Laboratory Standards (NCCLS) using cation supplemented Mueller-Hinton broth and 2.5% laked horse blood. MICs were read after incubation (18-20 hours at 35°C) and recorded as the lowest concentration of antimicrobic that inhibits visible growth. Antibiotics used in testing included ampicillin, apramycin, ceftiofur, chlortetracycline, clindamycin, enrofloxacin, erythromycin, florfenicol, gentamicin, neomycin, oxytetracycline, penicillin, spectinomycin, sulfachloropyridazine, sulfadimethoxine, sulphathiazole, tiamulin, tilmicosin, trimethoprim/sulphamethoxazole, and tylosin.

Results

Isolation In trial 1, *L. monocytogenes* was presumptively identified overall by the TaqMan assay in 0.57% of samples (12 of 2,122 samples). Although fecal and rectal contents were negative, 4.47% of caecal content samples (8 of 179) yielded presumptive *L. monocytogenes*. Recoveries were also made from two tonsil samples (1.1%, 2 of 181 samples), a carcass before the organic acid rinse (0.56%, 1 of 179 samples) as well as the combined ventral thoracic and subiliac lymph nodes (0.55%, 1 of 181 samples).

In the second trial, overall *L. monocytogenes* was presumptively identified in 0.14% of samples (1 of 736 samples) by a multiplex PCR assay that relies on gel detection. A single isolate was recovered from a meat block sampled during the fourth testing period. *L. monocytogenes* was not isolated from lymph nodes, tonsils, or carcass swabs. In trial 2, other species of Listeria were recovered in caecal contents (20%), and environmental swabs (14.6%) and infrequently from lymph nodes (<1%) and carcass swabs (1.88%).

Serotyping Only isolates presumptively identified as *L. monocytogenes* in trial 1 only were serotyped and were assigned to either serotype 1a or 4b factor 6.

Virulence assays Only presumptive *L. monocytogenes* as well as other species of Listeria recovered in trial 1 were evaluated in a virulence assay. Seven strains were highly virulent (>90% killing at 6 hours) and were thus confirmed as *L. monocytogenes*. These isolates were obtained from a tonsil (2 isolates), a carcass swab before the organic acid rinse (2 isolates), and two meat blocks (3 isolates). Isolates that were moderately or avirulent (<50% killing at 6 hours) in the hybridoma assay were identified as other Listeria species.

Antimicrobial testing Only presumptive *L. monocytogenes* isolates recovered in trial 1 were tested against 20 antibiotics and exhibited 16 different resistance patterns. All isolates were resistant to at least one antibiotic. The average isolate was resistant to 6.91 antimicrobials. All of the isolates were resistant to tiamulin, and none of isolates were resistant to ampicillin, penicillin, or trimethoprim/sulphamethoxazole.

Discussion

The goal of this study was to determine the distribution of *L. monocytogenes* in cull sows. Two trials were conducted at a single Midwest packing plant in 2001 (n=179 cull sows, 2,122 samples) and in 2002 (n= 160 cull sows, 736 samples). Fecal samples collected antemortem (trial 1) as well as animal tissues, carcass, and environmental swabs collected at the abattoir (trials 1 and 2) were analyzed. When results from both trials were combined, overall *L. monocytogenes* was presumptively identified in 0.45% of the total samples (13 of 2,858). After confirmation by conventional methods, virulence

testing, and serotyping, overall eight isolates from a total of five (0.17%) samples (n=2,858), including tonsils, carcass swabs, and meat blocks from both trials, were confirmed as *L. monocytogenes*.

In a previous study of ~300 young market weight hogs, we recovered *L. monocytogenes* from 2.5% of hog tissues (n=1,849). *L. monocytogenes* was also cultured from ~50% of ground pork (n=340 samples), which was prepared from hogs slaughtered on the previous day and whose carcasses were not sampled. The ground pork was produced in the plant on the same day that we were sampling the study hogs. In this earlier study, the increase in recovery of *L. monocytogenes* from the whole carcass to processed pork indicated contamination during processing.

In the current study of the cull sow, *L. monocytogenes* was recovered infrequently. The lower recovery from tissues at slaughter may be due to the fewer number of cull hogs slaughtered daily (~500) compared with market weight younger hogs slaughtered hourly (~1,100). The slower line speed and the lower volumes of water used during slaughter resulting in a drier abattoir environment may also explain the lower recovery in cull sows. In this current study, meat block samples were processed the same day and derived from the sows for which carcass data were obtained. No ground pork was made at the plant. In the previous study, ground pork was sampled the day after slaughter after carcasses had been held in the air chiller. Further, in the previous study ground pork originated from hogs other than those initially screened for Listeria.

In conclusion, these results when combined with data obtained earlier for younger market weight hogs indicate that *L. monocytogenes* occurs infrequently in live animals and yet may be found in the pork product. This again suggests contamination during processing.

Acknowledgements

We are grateful to Sharon Franklin and Carol Wiltsey for technical assistance. We acknowledge financial support of the Iowa State University Food Safety Consortium.

References

1. Bhunia, A.K., P.J. Steele, D.G. Westbrook, L.A. Bly, T.P. Maloney, and M.G. Johnson. 1994. A six ñhour in vitro virulence assay for Listeria monocytogenes using myeloma and hybridoma cels from miurine and human sources. Microb. Pathog. 16:99-110.
2. Duffy, E. A., K. E. Belk, J. N. Sofos, G. R. Bellinger, A. Pape, and G. C. Smith. 2001. Extent of microbial contamination in United States pork retail products. J Food Prot. 64:172-178.
3. Goulet, C., J. Rocourt, I. Rebiere, C. Jacquet, C. Moyse, P. Dehaumont, G. Salvat, and P. Veit. 1998. Listeriosis outbreak associated with the consumption of rillettes in France in 1993. J Infect Dis. 177:155-160.
4. Jacquet, C. B., R. Catimel, R. Brosch, C. Buchreiser, P. Dehaumont, V. Goulet, A. Lepoutre, P. Veit, and J. Rocourt. 1995. Investigations related to the epidemic strain involved in the French listeriosis outbreak in 1992. Appl Environ Microbiol. 61:2242-2246.
5. Kanuganti, S., I.V. Wesley, P.G. Reddy, J. Mckean and H.S. Hurd. 2002. Detection of Listeria monocytogenes in pigs and pork. J Food Prot.65:1470-1474.
6. Nogva, H.K., K. Rudi, K. Naterstad, A. Holck, and D. Lilehaug. 2000. Application of 5ínuclease PCR for quantitative detection of Listeria monocytogenes in pure cultures, water, skim milk, and unpasteurized whole milk. Appl Environ Microbiol. 66:4266-42271.
7. USDA-Food Safety and Inspection Service. 1996. Nationwide pork microbiological baseline data collection program: market hogs, April 1995-March 1996. U.S. Department of Agriculture, Food Safety and Inspection Service, Science and Technology, Microbiology Division, Washington, D.C.
8. Wesley, I. V. 1999. Listeriosis in animals, p. 39-74. In E. T. Ryser and E. H. Marth (eds.), Listeria, Listeriosis and Food Safety. Marcel Dekker, Inc., New York.
9. Wesley, I. V., K. M. Harmon, A. Ramos, and J. Dickson. 2001. Application of a multiplex PCR assay for the simultaneous confirmation of Listeria species and Listeria monocytogenes. J Food Prot. 65:780-785.