Low Prevalence of *Listeria monocytogenes* in Cull Sows and Pork

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ABSTRACT

The goal of this study was to determine the prevalence of *Listeria monocytogenes* in sows slaughtered at a single Midwestern plant on two occasions (trial 1, n = 179 sows; trial 2, n = 160 sows). Fecal samples collected antemortem (trial 1) as well as animal tissues, and carcass swabs collected at the abattoir (trials 1 and 2) were analyzed. Eight isolates of *L. monocytogenes* were recovered from five samples that represented 0.18% of the total samples (n = 2,775). In trial 1, *L. monocytogenes* was detected in a tonsil sample (0.6%; 1 positive of 181 tonsils), in a carcass (0.6%; 1 positive of 179 carcasses), which was sampled prior to the organic rinse, and in two chopped meat block samples (1.2%; 2 positive of 165 samples). In trial 2, *L. monocytogenes* was only detected in a single chopped meat block sample (0.15%; 1 positive of 688 total samples). These data indicate the low prevalence of *L. monocytogenes* in the cull sow.

*Listeria monocytogenes* accounts for ~2,518 human cases, 2,322 hospitalizations, and 504 deaths (~21% fatality case rate) annually in the United States (17). The Centers for Disease Control and Prevention have targeted the reduction of human listeriosis from the 1987 baseline of 0.7 cases per 100,000 to 0.25 cases per 100,000 population by the year 2010 (www.healthypeople.gov). FoodNet, which continuously monitors seven bacterial foodborne pathogens in 10 states, representing 44.5 million people or 15% of the U.S. population, has documented a 32% decline in human listeriosis from 1996, indicating that the projected reductions could be achieved (5). Listeriosis recalls and outbreaks have involved pork products (6, 10, 12). From 2000 to 2006, in the United States, 51% of class I (voluntary) recalls were attributed to *L. monocytogenes* contamination of pork (www.fsis.usda.gov/fsis.recall). The U.S. Department of Agriculture (USDA) Food Safety Inspection Service (FSIS) nationwide microbial baseline survey of hogs reported *L. monocytogenes* on 7.4% of hog carcasses (24). A survey in Taiwan reported a lower prevalence of 0.7% for 1,650 hog carcasses (29).

Although the origin of contamination in the pork abattoir is unclear, low levels of *L. monocytogenes* introduced by the live hog into the processing plant may amplify in the environment and subsequently contaminate carcasses and meats with increasing frequency during processing, especially during fabrication of ground pork (3, 11, 21, 22). In a survey of pigs in Bosnia and Herzegovina, *L. monocytogenes* was isolated from swine lymph nodes (5%), pig meat (8%), and in 9% of samples collected from bleeding, scalding, and evisceration areas (16). Abattoir workers assigned to these locations as well as carcasses sampled at these sites harbored *L. monocytogenes*. In an earlier study of young market weight hogs, we did not isolate *L. monocytogenes* from fecal samples, but recovered it on 4% of carcasses and in 22% of pork from unrelated hogs freshly ground that day at the slaughterhouse (14).

Antemortem testing of animals for *Listeria* relies on fecal as well as tonsil sampling. In Europe, fecal carriage rates range from 0% in the United Kingdom to nearly 47% of hogs sampled in Hungary (8, 21, 25–27). The high infection rate of up to 60% recorded for tonsils, where *L. monocytogenes* may be recovered more frequently than from feces, indicates that this sampling site may be an accurate predictor of carrier status and also that swine may be important reservoirs of *L. monocytogenes* (22, 27).

Because healthy carrier hogs may be the most probable source of *L. monocytogenes* contamination in the plant environment, reducing the carriage in hogs may reduce human foodborne listeriosis. The on-farm reduction of foodborne pathogens, such as *L. monocytogenes* spp., in hogs has focused on young market weight swine (~265 lb [~120 kg]), which represent ~95% of the annual slaughter pig inventory in the United States. Whereas primal cuts are derived mainly from market weight hogs, nearly 100% of whole hog sausage meat, including pepperoni and specialty sausages, is derived from either cull sows or boars. Few studies have recorded the prevalence of *L. monocytogenes* in the older hog (1). Whether farm management practices for

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sows versus market weight hogs, including animal housing densities, contact with other hogs facilitating transmission, or veterinary care provided the breeding animal, caused these differences is unknown.

Our goal was to determine whether the cull sow (~500 lb) with a longer life expectancy (three to six parities) poses a greater risk for L. monocytogenes contamination of pork when compared with our earlier study of younger market weight hogs (170 to 180 days of age at slaughter) (14).

**MATERIALS AND METHODS**

**Cull sows.** Two trials were conducted in the Midwestern region of the United States. The first consisted of five sampling periods (n = 40 hogs per period) from January to March 2001. Individually housed sows from multiple farms were delivered to a buying station, selected on basis of body condition scores and weight limits set by the slaughter plant and transported to the abattoir, as described (15). Fecal sampling at the buying station prior to loading, transportation (720 km; ~10 hours) to the abattoir, and order of slaughter to assure batch integrity, and samples collected at slaughter (tonsil swabs, ileocecal lymph nodes, ventral thoracic lymph nodes, subiliac lymph nodes, cecal contents, transverse colon contents, and carcass swabs) have been described (15). The second trial consisted of four sampling periods (n = 40 sows each) from February to April 2002. No antemortem sampling was attempted for trial 2 due to the low recoveries in trial 1. At slaughter, ileocecal lymph nodes, subiliac lymph nodes, cecal contents, precarcass wash sponge swabs (Nasco, Ft. Atkins, Wis.) of the left and right carcass section using the standard three-site USDA-FSIS procedure (300 cm²), and chopped meat block samples were collected as described (15). Because of the size of the animals (~500 lb [~227 kg]), the safety issues raised in obtaining these samples, and the lack of isolation of Listeria in the first trial, no tonsils were sampled in trial 2. Thoracic lymph nodes were not analyzed for trial 2. All samples were placed in Whirl-Pak plastic bags (Nasco) and transported on ice to the National Animal Disease Laboratory (Ames, Iowa).

**Microbiological testing.** Samples were cultured for L. monocytogenes as described previously (14). At least two colonies exhibiting typical Listeria appearance were picked from each PALCAM plate and subcultured to trypticase soy agar slants for confirmation by multiplex PCR, as described (28).

**DNA extraction procedures.** A loopful of bacterial growth on PALCAM agar was resuspended in 150 μl of sterile distilled water in a microcentrifuge tube. Cells were lysed by boiling (110°C, 10 min), the suspension pelleted (Eppendorf microcentrifuge model 5417C) and placed on ice. An aliquot of the supernate (5 μl) was used as the DNA template for PCR amplification.

**PCR amplification.** The primers and amplification conditions used in this study were described earlier (28). Isolates were identified as L. monocytogenes based on the presence of the both the 938- and 174-bp amplicons; the presence of a single 938-bp product was characteristic of Listeria innocua (28). In trial 1, confirmed L. monocytogenes strains were serogrouped, tested for antimicrobial susceptibility as well as virulence potential, and characterized by pulsed-field gel electrophoresis (PFGE). Because of the low recovery of L. monocytogenes in trial 1, efforts were expanded in trial 2 to also identify L. innocua.

**Serotyping.** L. monocytogenes isolates were typed using the slide agglutination test for serogroups 1 and 4, as described (2).

**Antimicrobial susceptibility testing.** MICs were determined using a microdilution procedure (Sensititre, Trek Diagnostic Systems, Cleveland, Ohio) as stated in the National Committee for Clinical Laboratory Standards using cation-supplemented Mueller-Hinton broth and 2.5% laked horse blood. Isolates were scored as susceptible, intermediate, or resistant, as described by SWIN Reference Manager (Trek Diagnostic Systems).

**Virulence testing.** The virulence of seven isolates of L. monocytogenes was determined in the 6-h hybridoma cytotoxicity assay as described (18). All tests were repeated three times.

**PFGE.** Isolates were processed according to the standardized protocol set forth by the Centers for Disease Control and Prevention for L. monocytogenes in the procedural manual for laboratories participating in the PulseNet program (4). The captured image was analyzed using Molecular Analyst Software (Applied Maths, Sint-Martens-Latem, Belgium), and a dendrogram was generated using the Dice coefficient.

**RESULTS**

Isolation. As summarized in Table 1, L. monocytogenes was confirmed in 0.19% of samples (4 of 2,087) in trial 2. More specifically for trial 1, recoveries were made from one tonsil sample (0.6%, 1 of 181 samples; isolates 184A, 184B), from a carcass prior to the organic acid rinse (0.1%, 1 of 179 carcasses; isolates 199A, 199B), and from 2 of the 165 chopped meat samples (1.21%, 2 positive of 165 samples; isolates GE 1-4A, GE 1-4B, and GE 1-3B). Feces as well as rectal and cecal contents were negative as were ventral thoracic, ileocecal, and subiliac lymph nodes.

In the second trial, as summarized in Table 1, L. monocytogenes was identified in 0.15% of samples (1 positive of 688 samples). The single isolate was recovered from a meat block sampled during the fourth sampling period. In trial 2, the survey was expanded to incorporate L. innocua, which were subsequently recovered in cecal contents (20%), and environmental swabs (14.6%), and infrequently from lymph nodes (<1%) and carcass swabs (1.9%).

Serotyping. The seven isolates confirmed as L. monocytogenes in trial 1 were available for serotyping. Isolates from the single tonsil sample (isolates 184A, 184B) and single precarcass swab (isolates 199A, 199B) were assigned to serotype 4b, factor 6. The isolates from the two chopped meat samples (isolates GE 1-4A, GE 1-4B, and GE 1-3B) were assigned to serotype 1/2a, factor 1.

Antimicrobial testing. The seven isolates verified as L. monocytogenes from trial 1 were tested against 20 an-
TABLE 2. *In vitro* Ped-2E9-based tissue culture virulence assay results for seven *Listeria* isolates

<table>
<thead>
<tr>
<th>Isolate designation</th>
<th>Description</th>
<th>% hybridoma cell death</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>184A Tonsils 100.0/H11006</td>
<td>Highly virulent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>184B Tonsils 100.0/H11006</td>
<td>Highly virulent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GE 1-3B Meat block 99.3/H11006</td>
<td>Highly virulent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GE 1-4A Meat block 4b 99.5/H11006</td>
<td>Highly virulent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GE 1-4B Meat block 4b 99.5/H11006</td>
<td>Highly virulent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>199A Precarcass swab wash right side 92.5/H11006</td>
<td>Highly virulent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>199B Precarcass swab wash right side 92.2/H11006</td>
<td>Highly virulent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 L. <em>monocytogenes</em> (V7), serotype 1/2a (positive control) 96.3/H11006</td>
<td>Highly virulent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 L. <em>monocytogenes</em> (Scott A), serotype 4b (positive control) 98.1/H11006</td>
<td>Highly virulent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>163 L. <em>monocytogenes</em> (NADC), serotype 4b (positive control) 98.9/H11006</td>
<td>Highly virulent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 L. <em>innocua</em> (negative control) 11.8/H11006</td>
<td>Avirulent</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* Mean of three tests with two replicates per test.

Virulence assays. The results of virulence testing based on the hybridoma cytototoxicity assay are summarized in Table 2. The seven isolates confirmed as *L. monocytogenes*, which were recovered in trial 1, were highly virulent (>90% killing at 6 h).

**PFGE.** *L. monocytogenes* isolates recovered from trial 1 were compared by PFGE. As shown in Figure 1, isolates from the meat blocks (isolates GE 1-4A, GE 1-4B, and GE 1-3B) were highly similar but clearly unrelated to the two isolates (A and B) obtained from either the carcass (isolates 199A, 199B) or tonsil (isolates 184A, 184B) swab.

**DISCUSSION**

To estimate the prevalence of *L. monocytogenes* in sows, two trials were conducted at a single Midwest packing plant, which slaughtered only culled breeders. When results from both trials were combined, five samples (0.1%; 5 positive of 2,775 samples), including tonsils (one tonsil, two isolates), carcass swabs (one swab, two isolates), and meat samples (three blocks, four isolates) from both trials, harbored *L. monocytogenes*.

Prevalence estimates for *Listeria* in hogs are based on recovery from tonsils, feces, as well as carcass swabs and lymph nodes obtained at slaughter (1, 14, 19, 24, 27). In this study, *L. monocytogenes* was cultured from tonsils (0.6%; 1 positive of 181 samples) but not from feces (0%; 0 positive of 302 samples). In our previous study of younger market weight hogs (14), we detected *L. monocytogenes* in tonsil scrapings collected on-farm (0.3%; 1 positive of 297 samples) but not in rectal samples taken at slaughter (0%; n = 255 samples). Reported recoveries from tonsils range from 0 to 61%, which may reflect differences in tonsils sampling methods, age of pigs surveyed, season (especially spring and winter when *L. monocytogenes* prevalence is the highest), herd size, farm management practices, and geographic differences (21–23, 27, 28).

A source of *L. monocytogenes* contamination of carcasses is assumed to be the intestine, which may rupture at slaughter (19). In the current study of culled sows, as in our previous study of younger market weight animals (14), *L. monocytogenes* was not cultured from intestine. In contrast, *L. monocytogenes* has been recovered from 0 to 47% of swine feces especially in Eastern Europe (8, 16, 21, 22, 25–27). Husbandry practices such as feeding pigs dry feed or...
silage, as customary in Eastern Europe, age at slaughter, differences in housing, biosecurity, and regional differences, since prevalence is reportedly higher in northern climates, may account for some of the variation in the prevalence of *Listeria* in healthy pig feces (19, 21, 22, 26).

*L. monocytogenes* was recovered infrequently from live cull sows or from their tissues including ileocecal, subiliac, or thoracic lymph nodes during these trials, which were conducted in the winter and spring, which favors the recovery of *Listeria* in ruminants (27). In our previous study of ~300 young market weight hogs conducted in the Midwest during the summer months, we recovered *L. monocytogenes* from 2.5% of hog tissues (*n* = 1,849), including thoracic (3.5%; 9 positive of 259 samples) and superficial inguinal lymph nodes (1.9%; 5 positive of 262 samples). Using these same isolation protocols, we earlier identified higher levels of *L. monocytogenes* in ground pork (14).

In the current study, a single isolation was made from a single cull sow carcass sampled during the two trials (0.3%; 1 positive of 339 carcasses). This is less than the 7.3% prevalence of *L. monocytogenes* on hog carcasses, which was achieved during the USDA-FSIS baseline study (23). In our previous survey of younger market weight hogs 11 (4.1%) of 267 carcasses yielded *L. monocytogenes* (14). Differences in *L. monocytogenes* recovery at slaughter may reflect higher animal housing densities for younger market animals versus sows, which, with a range of two to six parities, are housed individually. Slaughter practices for the younger animal versus the older sow may also contribute. To illustrate, fewer culled hogs are slaughtered daily (~500) when compared to the number of market weight younger hogs slaughtered hourly (~1,100). The resultant slower line speed and consequently the lower volumes of water used during slaughter yielding a drier abattoir environment may also lower the frequency of *L. monocytogenes* on the sow carcasses. It is also possible that the abattoir chosen for this study purchased breeders of the highest quality, thereby minimizing the prevalence of *L. monocytogenes*. Clinical listeriosis in hogs in the Midwest is rare. In Iowa, a major hog-producing state, from 1993 to 2000, of a total of 253 listeriosis submissions to the state veterinary diagnostic laboratory, none were from pigs (27).

In this current study, the chopped block meat samples were processed the same day and derived from the sows for which carcass data were obtained. For the two trials, a total of 213 meat blocks were sampled. Of these, three chopped meat samples (1.4%) yielded *L. monocytogenes*. The limited number of chopped meat samples contaminated with *L. monocytogenes* reflects low levels of carcass contamination as well as plant hygiene.

Serootypes 1/2a, 1/2b, and 4b are the major groups associated with human listeriosis (20). Seven isolates, all from trial 1, were assigned to serotype 1/2a (meat block samples) and serotype 4b (tonsil and carcass samples) and killed >90% of cells in the 6-h hybridoma cytotoxicity assay reflecting their virulence potential.

*L. monocytogenes* is routinely sensitive to penicillin, gentamicin, and tetracycline, the drugs of choice, as well as cefotaxin, erythromycin, and trimethoprim/sulfonamide (13). The seven *L. monocytogenes* isolates were resistant to tiamulin, which is used to treat diseases of hogs, including proliferative ileitis (*Lawsonia intracellularis*), pleuropneumonia (*Actinobacillus pleuropneumonia*), and respiratory tract pathogens (www.tiamutin.com/vet-pig/enteric/ileitis/en/microbio.shtml). Resistance to chlorotetracycline, which is the recommended antimicrobial to treat bovine listeriosis (28), was also noted. Health histories including drug treatments and specifically whether the sows were administered either tiamulin or tetracycline are unknown.

Identity of PFGE profiles has shown that processing equipment contaminates pork and vice versa (1, 9, 22). By PFGE analysis of *L. monocytogenes* isolated during trial 1, three isolates from the meat blocks were highly similar but clearly unrelated to those obtained from either the carcass or tonsil swabs. In the absence of environmental testing in trial 1, we cannot determine the origin of *L. monocytogenes* recovered on the carcass or meat blocks or appraise plant hygiene.

In summary, these results when compared with data obtained earlier for younger market weight hogs suggest that sows despite their longer life span do not pose additional public health risks to the food chain.

ACKNOWLEDGMENTS

We are grateful to the pork producers who granted access to their hogs for preslaughter testing. We thank the management of the packing plant for access to their facility. The technical skills of Dr. Jean Lauber, Sharon Franklin, Laura Byl, and Carol Wiltsie are appreciated. We are indebted to Linda Miller for the secretarial expertise. We acknowledge financial support of the Iowa State University Food Safety Consortium and the generosity of the U.S. Department of Agriculture–Agricultural Research Service–National Alliance for Food Safety.

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