

Research Note

Tracking the Sources of *Salmonella* in Ground Beef Produced from Nonfed Cattle

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ABSTRACT

The objective of this study was to determine the source(s) of *Salmonella* contamination in ground beef. One hundred dairy cows were harvested in a U.S. commercial beef processing plant. Samples of hides, carcasses after hide removal and before exposure to antimicrobial intervention, carcasses after all antimicrobial interventions, superficial cervical lymph nodes from the chuck, trim, ground beef, and air were obtained. Ninety-six percent of the hide samples, 47% of the carcasses before intervention, 18% of the lymph nodes, 7.14% of the trim, and 1.67% of the ground beef samples were positive for *Salmonella*. None of the samples obtained from the carcasses after the full complement of interventions and none of the air samples were positive for *Salmonella*. All *Salmonella*-positive samples were subjected to pulsed-field gel electrophoresis, and eight DNA *Xba*I restriction patterns were identified. The majority of isolates had one of two restriction digest patterns. The strain isolated from ground beef had the same pattern as the strains isolated from hides and from carcasses immediately after hide removal. The *Salmonella* isolates from trim samples and lymph nodes also had the same restriction digest pattern. These results indicate that hide and lymph nodes are the most likely sources of *Salmonella* in ground beef. Dressing practices that effectively reduce or eliminate the transfer of bacteria from hide to carcass and elimination of lymph nodes as a component of raw ground beef should be considered as measures to reduce *Salmonella* contamination of ground beef. Because total elimination of lymph nodes from ground beef is not possible, other approaches should be explored. Easily accessible lymph nodes could be screened for *Salmonella* very early in the slaughter process. When the results are positive for *Salmonella*, the corresponding carcasses should be fabricated separately at the end of the production run, and the trim from these carcasses should be subjected to a treatment that destroys *Salmonella*.

Salmonellosis continues to be a major foodborne illness in the United States. In 2009, *Salmonella* accounted for 7,039 (40.3%) of the 17,468 laboratory-confirmed cases of foodborne infection (8). *Escherichia coli* O157:H7 is considered an adulterant in trim and ground beef, but *Salmonella* is not. However, in 2009, for the first time more ground beef was recalled because of the presence of *Salmonella* than because of the presence *E. coli* O157 (16). Since 2000, at least four food recalls have been due to contamination of ground beef with *Salmonella* (16). Depending on the immune status of infected individuals and the dose of ingested *Salmonella*, the mere presence of *Salmonella* can pose a public health risk. The infectious dose of the organism differs by strain and host susceptibility and has been reported as 15 to 20 cells (19).

In 1999, the U.S. Department of Agriculture (USDA) Economic Research Service estimated the annual cost due to foodborne *Salmonella* infection in the United States at \$2.3 billion (9). Persons infected with *Salmonella* usually recover; however, some infected individuals require

hospitalization. Antibiotics may be part of the treatment of hospitalized individuals. Thus, recent emergence of multi-drug-resistant (MDR) *Salmonella* strains is a significant food safety concern.

Salmonella reservoirs including the environment, produce, reptiles, soil, and meat animals. The estimates for national *Salmonella* prevalence are 1.27% of beef trim (18) and 4.2% of ground beef (5). Identification of the sources of *Salmonella* should enable beef processors to limit its prevalence in ground beef. Hide has been identified as a major source of foodborne pathogens, including *Salmonella* (3, 4, 11). *Salmonella* also has been found in cattle lymph nodes (2, 13, 15). Most or all of the lymph nodes located in fat tissues of beef carcasses are not removed during fabrication. These lymph nodes are ground with lean and fat trimmings to produce ground beef, making lymph nodes a possible source of *Salmonella* in ground beef. None of the published studies have included the simultaneous determination of *Salmonella* on the hides and in lymph nodes or sampling and tracking of all potential sources of *Salmonella* (by implication, MDR *Salmonella*) in ground beef. The objective of this study was to use pulsed-field gel electrophoresis (PFGE) to profile *Salmonella* isolates from

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all possible cattle sources (air in the processing plants, hides, carcass, trimmings, and lymph nodes) to identify the possible source(s) of *Salmonella* contamination in ground beef.

MATERIALS AND METHODS

Sampling locations. Samples were collected from 100 dairy cows from exsanguination through production of ground beef in a medium-size (fewer than 1,000 head per day) U.S. commercial beef processing plant. Sampling sites included hide, carcass after hide removal, and carcass after a full complement of antimicrobial interventions. Interventions included a carcass hot water wash and a subsequent lactic acid spray wash in an automated spray cabinet; lactic acid was applied by hand to areas of the carcasses that were inaccessible in the automated cabinet.

Hide and carcass samples were collected as described by Arthur et al. (1). After overnight chilling, superficial cervical lymph nodes from one side of the carcass were removed from the chuck. Chuck lymph nodes were selected because they are located in tissues likely to be used in ground beef production. The 100 carcasses were then fabricated as a group, separate from all others, and all trimmings generated from these carcasses were collected and sampled. Consistent with the establishment's food safety practices, trimmings were sampled and tested for the presence of *E. coli* O157:H7 by a third party laboratory.

Trim from carcasses was collected into a standard bin that holds approximately 910 kg. Trim from the 100 carcasses filled 14 combo bins. Each combo bin was sampled using an $N = 60$ excision equivalent method (12). All trim samples were negative for *E. coli* O157:H7. Approximately 48 h after slaughter and at the beginning of the production day, trim in the 14 combo bins was ground and sampled; this was the only source for ground beef. Grinding of the approximately 12,740 kg of trim took 75 min. Approximately every 90 s, a 325-g sample of ground beef was collected as the beef exited the grinder. Periodically, the process was stopped to remove fat and cartilage from the grinder head. Every time the grinder head was cleaned and at the end of the process, samples of fat and cartilage from the grinder head were collected, for a total of 10 such samples. Air samples were also collected at each sampling location (hides-on and hides-off sides of the harvest floor, chillers, and fabrication and grinding areas). All samples were shipped in containers with cold packs to IEH Laboratories and Consulting Group (Lake Forest Park, WA) for analysis. All samples were analyzed for the presence of *Salmonella*, and all *Salmonella* isolates were analyzed by PFGE.

Sample collection. Whirl-Pak sterile sponges (Nasco, Fort Atkinson, WI) were used for swabbing carcass and hides. Samples of trim, ground beef, and lymph node samples were collected into Whirl-Pak filter bags. Before sampling, sponges were aseptically hydrated with 25 ml of Butterfield's phosphate buffer (1.25 ml/liter 6.8% KH_2PO_4 , pH 7.2, stock solution in distilled water). Hide samples were obtained by swabbing a 1,000-cm² area over the plate region. The leading sides of the carcasses were sampled immediately after hide removal (before application of any antimicrobial intervention), and the trailing sides were sampled after the application of all interventions. For carcass samples, one sponge was used to swab approximately 4,000 cm² of the navel, plate, brisket, and shank areas. Another sponge was used to sample approximately 4,000 cm² of the inside and outside round. These two sponges were subsequently combined to make a single sample.

At each air sampling site, 500 liters of air was collected with an air sampling device (ABLE Air Sampler, IEH) and directed onto a RODAC plate (BD, Franklin Lakes, NJ) containing 5 ml of

sterile brain heart infusion agar (BD). The air sampler was constructed according to basic physical principles governing the design of impact air samplers, as discussed by Yao and Mainelis (20). The collection efficacy of impact samplers is a function of the velocity of air passing through perforations in a disk and the disk-to-medium collection plate distance. The velocity of the air sampler was computed as 29 m/s based on the design characteristics of sampling air flow rate (100 liters/min), number of perforations (73), and perforation diameter (1.2 mm) according to equation 1 of Yao and Mainelis (20). In combination with a disk-to-plate distance of 3 mm, this design yields high relative overall collection efficiency (see Fig. 2 of Yao and Mainelis (20)). The air sampler was designed with a motor speed controller that uses a feedback loop to maintain a constant number of revolutions per minute, ensuring uniform air flow. A built-in timer ensures that sample collection times, and therefore total volumes of air collected, are uniform between samples. The sample time can be selected by the user but typically is set at 5 min so the total volume of air sampled is 500 liters. To provide a flow rate of 100 liters/min, all IEH air samplers were calibrated with a standard vane anemometer (Extech Instruments, Tampa, FL) inserted into a special hood, which fits over the top of the air sampler and directs all air flow through the anemometer. RODAC plates were then placed in sterile Whirl-Pak bags and shipped to the laboratory.

Sample processing. Upon arrival at the laboratory, samples were processed according to sample type. For sponge samples, 25 ml of prewarmed IEH multiplex *E. coli* medium (<http://www.iehinc.com/PDF/IEH%20Multiplex%20E.coli%20%20Medium.pdf>) was added to each sample bag, and samples were incubated at 35°C. After 24 h of incubation, 2-ml aliquots were taken from the enriched samples and frozen in 10% glycerol. A 1-ml aliquot was processed for *Salmonella*. Trim and ground beef samples were weighed, and three volumes of medium were added and stomached for 1 to 2 min. Bags of enriched samples were incubated at 35°C for 24 h, and then 2-ml aliquots of the enriched samples were removed for PCR processing and frozen in 10% glycerol. For lymph node samples, excess fat and fascia were aseptically trimmed, sterilized in a boiling water bath for 3 to 5 s, and then cut into multiple pieces. The lymph node pieces were placed into a filtered stomacher bag (Nasco), weighed, and pulverized with a rubber mallet. Three volumes of enrichment medium were added to the pulverized lymph node samples, which were then stomached and incubated as above.

Screening for *Salmonella*. The presence of *Salmonella* was determined by using the IEH *E. coli* O157, Stx-producing *E. coli* (STEC) with intimin, and *Salmonella* test system (AOAC 100701) as outlined by Stopforth et al. (14). Samples that were positive for *Salmonella* in this multiplex PCR assay were plated onto xylose lysine Tergitol (XLT) 4 agar and brilliant green sulfadiazine agar and processed following the USDA Food Safety and Inspection Service MLG 4.04 procedure (17). Samples that produced a negative result in this PCR assay were inoculated into Rappaport-Vassiliadis broth and tetrathionate broth, analyzed by PCR assay as above, and processed following the MLG 4.04 procedure.

Isolation and identification of *Salmonella*. Colonies with culture characteristics typical of *Salmonella* on XLT4 (black or red colonies with or without black centers) were picked and restreaked on XLT4 for isolation and incubated at 35°C for 24 to 48 h. Pure cultures were streaked onto tryptic soy agar plates containing 5% sheep blood (BBL, BD, Sparks, MD), incubated at 35°C for 24 h, and identified using the Vitek Identification System (bioMérieux, Marcy l'Etoile, France).

TABLE 1. Incidence of *Salmonella* in various samples obtained from 100 cattle

Sample type	No. of samples	No. of <i>Salmonella</i> -positive samples		
		PCR	USDA FSIS (17)	<i>Salmonella</i> prevalence (%)
Hide	100	95	96	96.0
Preintervention ^a	100	49	47 ^b	47.0
Postintervention ^c	50	0	0	0.0
Lymph nodes	100	18	18	18.0
Trim	14	1	1	7.14
Ground beef	60	1	1	1.67
Left over ^d	10	0	0	0
Air samples	33	0	0	0
Total	457	164	163	35.67

^a Samples obtained before antimicrobial intervention.

^b No *Salmonella* colonies were recovered from culture of two preintervention samples.

^c Samples obtained after all antimicrobial intervention.

^d Fat and cartilage removed from the grinder head at each cleaning time and at the end of the grinding operation.

PFGE. All isolates were analyzed by PFGE after *Xba*I restriction enzyme digestion according to the Centers for Disease Control and Prevention Pulse Net protocol for molecular subtyping of *Salmonella* (7). PFGE gel images were analyzed with BioNumerics software (Applied Maths, Inc., Austin, TX). The Dice similarity coefficient was used to calculate restriction fragment length polymorphism (RFLP) pattern similarity (with optimization at 0.5% and position tolerance at 1%), and the unweighted pair group method with arithmetic mean algorithm was used to construct a dendrogram. To determine the serovar of the *Salmonella* isolates, the RFLP patterns were compared with those of known *Salmonella* serovars in our database.

RESULTS AND DISCUSSION

Results obtained for *Salmonella* prevalence are reported in Table 1. Of the 457 samples tested, 164 samples were positive for *Salmonella* with the PCR assay and 163 were positive with the culture method. The highest prevalence was observed in samples obtained from hides (96%) followed by samples obtained from carcasses after hide removal (47%). None of the samples obtained from carcasses after the full complement of antimicrobial interventions were positive for *Salmonella*, but 7.14% of the trim samples obtained from these same carcasses were positive for *Salmonella*.

Several plausible explanations exist for why none of the samples obtained from carcasses were positive for *Salmonella* and yet the trimmings obtained from these carcasses were positive for *Salmonella*. First, to avoid repeated sampling of the same area, one carcass side was sampled immediately after hide removal and the other side was sampled after all antimicrobial interventions had been implemented; thus, the microflora might differ between sides. Second, although 8,000 cm² of the carcass was swabbed, this area represents at most only 25% of the carcass surface. Third, the final antimicrobial interventions used in the plant were hot water followed by lactic acid. These interventions may have injured but not killed *Salmonella* and other pathogens, and the time between exposure to intervention and collection of trim samples may have allowed these injured cells to recover. Deep cuts that

occur occasionally during the harvest process may have provided protection for *Salmonella* and other organisms from interventions designed to reduce or eliminate these pathogens. Although 18% of the lymph nodes, 7.14% of the trim samples, and 1.67% of the ground beef samples were positive for *Salmonella*, none of the air samples were positive for this pathogen. Thus, in this study air was not considered a mode of transmission for *Salmonella*.

The *Salmonella* prevalence data obtained in this study for hide, carcass before interventions, and carcass after interventions are similar to those previously reported (4, 6) for nonfed cattle (cull cows). The number of published reports on *Salmonella* presence in nonmesenteric lymph nodes is limited. Arthur et al. (2) found a 4.25% prevalence of *Salmonella* in lymph nodes from the chuck and flank of nonfed cattle, a value lower than the lymph node *Salmonella* prevalence reported here. Lymph nodes are part of the immune system, and among other functions they act as a filtering system for removing bacteria, viruses, and other foreign particles. Several researchers have reported the presence of various types of bacteria in mesenteric and nonmesenteric lymph nodes. Thus, it is not surprising to isolate *Salmonella* from lymph nodes. Likewise, because deep tissue lymph nodes are not removed during carcass processing into ground beef, *Salmonella* could easily be found in ground beef.

To determine the possible sources of the *Salmonella* isolates found in ground beef, all *Salmonella* isolates were subjected to PFGE analysis. When *Xba*I restriction patterns of DNA from each *Salmonella* isolate obtained from various samples (i.e., hide, preintervention carcass, postintervention carcass, lymph nodes, and meat) were analyzed by PFGE, eight restriction digest patterns (RDPs A through H) were observed. The majority of the isolates had patterns E or B (Fig. 1). Isolates from hides and preintervention carcass samples had multiple RDPs, whereas the lymph node, ground beef, and trim isolates each had a single unique RDP. The trim isolate had the same RDP as did the lymph node isolates (pattern F). The ground beef isolate had a pattern (B) common to preintervention carcass and hide

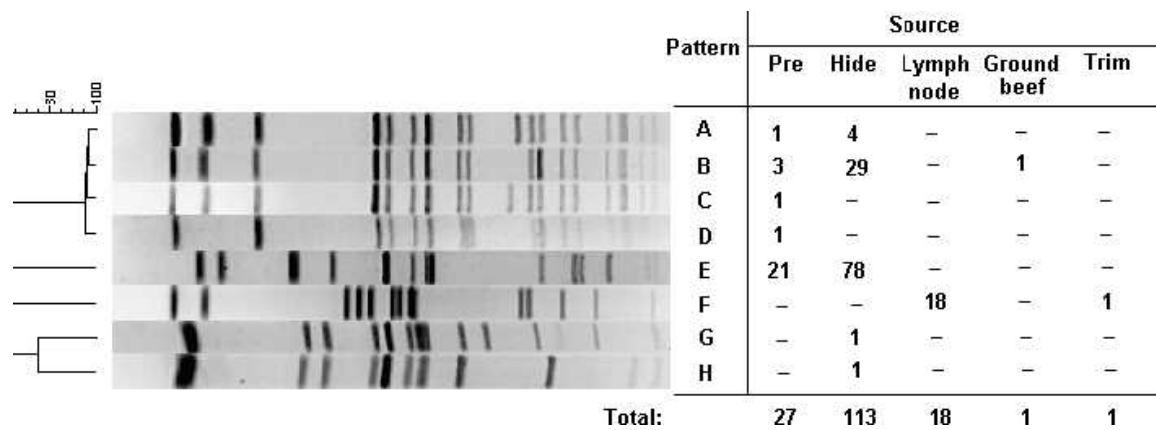


FIGURE 1. Relatedness of *Salmonella* isolates determined by pulsed-field gel electrophoresis of DNA fragments obtained with the *Xba*I restriction enzyme.

sample isolates. A database comparison determined that the RFLP patterns of the trim and lymph node isolates were similar to that of *Salmonella* Cerro and the RFLP patterns of the ground beef and hide isolates were similar to that of *Salmonella* Newport.

Samples of lymph nodes from 18 carcasses, one trim sample, and none of the ground beef samples contain *Salmonella* with RDP F. If these *Salmonella* isolates had been distributed throughout the superficial cervical lymph nodes and the lymphatic system in the 18 carcasses, three or four other large lymph nodes from each of these carcasses should also have been positive for this isolate. This number of lymph nodes (54 to 72), which weighed approximately 20 g each, potentially contributed 1 to 1.5 kg of contaminated material (or about 0.01%) to the total 12,700 kg of trim. In keeping with this estimate, *Salmonella* RDP F contamination was detected in one of the trim samples, which represented about 0.002% of the trim from the 14 samples collected and tested. The process of grinding this trim would be expected to have more evenly distributed the *Salmonella* with RDP F throughout the ground beef such that the group of 60 325-g samples tested (representing 0.15% of ground beef) should have included 10 or more *Salmonella*-positive samples. However, all the ground beef samples were negative for *Salmonella* RDP F. This finding suggests that the *Salmonella* in the lymphatic system may be concentrated in only a few lymph nodes, or at such a low level in contaminated lymph nodes that in either case it became diluted by grinding to the point where it was below the limit of detection of the PCR and culture methods used. An alternative explanation is that grinding does not evenly distribute *Salmonella* contamination to the level that can be detected by the sampling frequency used (one sample per minute, representing 0.15% of the total ground beef produced).

MDR *Salmonella* strains have emerged in recent decades. A number of food recalls have been due to contamination of ground beef with MDR *Salmonella* (16). The control of *Salmonella* requires a thorough understanding of its source and how the pathogen finds its way into ground beef. This study was conducted to determine the source(s) of *Salmonella* contamination in ground beef, thereby providing beef processors with the knowledge

required to reduce or eliminate *Salmonella* from the national ground beef supply. It is well established that hide is the main, if not the only, source of enteric pathogens in beef (10). During hide removal, a proportion of the bacteria on the hide is transferred to the carcass. The level of pathogen transferred is directly proportional to the efficacy of the dressing practices used in a given processing plant. Therefore, the level of bacteria on the carcasses after hide removal is a reflection of the plant's dressing practices. The purpose of all interventions used after dehiding is to remove as much of the transferred contamination as possible. Results of this study revealed that 96, 47, and 0% of the samples collected from the hide, the carcass after hide removal, and the carcass after interventions, respectively, were positive for *Salmonella*. Thus, although dressing practices might have been less than optimal, the interventions used in the plant were effective because all samples obtained from carcasses after the application of interventions were negative for *Salmonella*. However, the results for trim and ground beef indicate that interventions did not completely eliminate *Salmonella*, as 1 of the 14 trim samples and 1 of the 60 ground beef samples were positive for *Salmonella*. Because of the function of the lymph nodes in the animal's immune system and because deep tissue lymph nodes are not removed before the trim is ground, these lymph nodes probably are a source of *Salmonella*.

The results presented here indicate that hide and lymph nodes are the principal sources of *Salmonella* in ground beef. However, the design of this experiment did not allow determination of the relative contribution of hide versus lymph nodes to *Salmonella* contamination in the final ground beef product. Dressing practices designed to eliminate or minimize the level of *Salmonella* transferred from the hide to the carcass, interventions to remove *Salmonella* from the carcass, and lymph node removal should reduce or eliminate *Salmonella* from ground beef. However, the complete elimination of all lymph nodes is not possible because not all lymph nodes are accessible. Because lymph node removal is not practical and because lymph nodes are a potential source of *Salmonella* in ground beef, alternative strategies are needed. Further study is needed to determine whether carcasses with lymph nodes

that contain *Salmonella* can be detected reliably by testing of a sentinel node early in the slaughter process. If this testing is found to be feasible and accurate, then lymph nodes that are readily accessible on the harvest floor should be screened for the presence of *Salmonella* as early in the process as possible. Carcasses should be held and not fabricated until the results of the screening are known. When the results are positive for *Salmonella*, then the corresponding carcasses should be fabricated separately at the end of the production run, and the trim from these carcasses should be subjected to a treatment that destroys *Salmonella* before the trim is processed for consumption.

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