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Research Note

Prevalence and Characterization of *Salmonella* in Bovine Lymph Nodes Potentially Destined for Use in Ground Beef†

TERRANCE M. ARTHUR,* DAYNA M. BRICHTA-HARHAY, JOSEPH M. BOSILEVAC, MICHAEL N. GUERINI, NORASAK KALCHAYANAND, JAMES E. WELLS, STEVEN D. SHACKELFORD, TOMMY L. WHEELER, AND MOHAMMAD KOOHMARAIE

U.S. Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska 68933-0166, USA

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ABSTRACT

A potential source of pathogenic bacteria in ground beef is the lymphatic system, specifically the lymph nodes. Bacteria have been isolated from the lymph nodes of cattle at slaughter; however, most studies have dealt with mesenteric lymph nodes, which are not normally incorporated into ground beef. The objective of the current study was to determine the prevalence and multidrug-resistance status of *Salmonella* in bovine lymph nodes associated with lean and fat trimmings that might be utilized in ground beef production. Bovine lymph nodes (n = 1,140) were collected from commercial beef processing plants. Half of the lymph nodes sampled were obtained from cull cow and bull processing plants, and the remainder were obtained from fed beef processing plants. Lymph nodes located in chuck and flank adipose tissue were collected for this study. *Salmonella* prevalence in the lymph node samples was low, with an overall prevalence of 1.6% and a 95% confidence interval of 0.85 to 2.3%. Lymph nodes from cull cattle carcasses had a higher prevalence of *Salmonella* than did those from fed cattle carcasses. Lymph nodes from the flanks of cow and bull carcasses had the highest prevalence at 3.86%, whereas lymph nodes from the chuck region of fed cattle carcasses had the lowest prevalence at 0.35%. Three of the 18 *Salmonella*-positive lymph node samples contained multidrug-resistant *Salmonella,* and all 3 samples were from cull cattle.

Foodborne bacterial contamination of meat is responsible for several thousand illnesses per year in the United States *(12).* The primary means of reducing or preventing this type of disease is to identify the sources of contamination and minimize or remove the sources from the production process. Cattle hides have been consistently implicated as the main source of the foodborne pathogens that contaminate carcasses *(3, 4, 6, 13).* In response to this finding, various forms of antimicrobial interventions targeting cattle hides have been developed. These interventions have been implemented in commercial beef processing facilities and have been effective for reducing carcass contamination *(1, 7).* However, even after the implementation of these interventions, pathogenic bacteria are still found in ground beef, possibly indicating that other sources of these bacteria may be present.

One potential source of pathogenic bacteria in beef is the lymphatic system, specifically the lymph nodes. The lymphatic system is involved in immune function by acting as a filtering mechanism to sequester bacteria, viruses, and other foreign invaders for eventual destruction by lymphocytes. There are several reports of bacteria isolated from the lymph nodes of cattle at slaughter. In an earlier study, Lepovetsky et al. *(11)* found numerous bacterial species in bovine lymph nodes removed from the chuck and round regions. Sofos et al. *(20)* reported aerobic plate counts exceeding 1,000 CFU/g for one quarter of the mandibular and parotid lymph nodes they evaluated, but they did not detect *Salmonella* in any of the lymph nodes sampled. In two studies performed in Australia, *Salmonella* was isolated from several mesenteric lymph nodes, most notably the cecal and jejunal lymph nodes *(18, 19).* Samuel et al. *(19)* noted that although *Salmonella* was frequently isolated from the cecal and jejunal lymph nodes, no *Salmonella* was isolated from the duodenal lymph nodes of the same animals. This and similar observations led those authors to conclude that significant spread of *Salmonella* beyond the mesenteric lymph nodes does not occur.

Most of the studies performed to date have dealt with mesenteric lymph nodes, which would not be found in ground beef. The mesenteric lymph nodes are discarded during the evisceration process and do not present any food safety concerns. However, many lymph nodes are located within the fatty tissue of the beef carcass. Thus, lean and fat trimmings used in ground beef production may contain some lymph nodes. The objective of the present study was to determine the prevalence and multidrug-resistance status of *Salmonella* in bovine lymph nodes potentially destined for ground beef.

* Author for correspondence. Tel: 402-762-4227; Fax: 402-762-4149; E-mail: terrance.arthur@ars.usda.gov.

† Names are necessary to report factually on available data; however, the U.S. Department of Agriculture neither guarantees nor warrants the standard of the product, and the use of the name by the U.S. Department of Agriculture implies no approval of the product to the exclusion of others that may also be suitable.

MATERIALS AND METHODS

Sample collection. Bovine lymph nodes were collected from four commercial beef processing plants in the winter and spring of 2007. Lymph nodes were collected from two carcass locations, chuck and flank. Lymph nodes from these regions were utilized for this study because they are part of tissues that are likely to be used in ground beef production. The superficial cervical lymph node was obtained from the chuck, and the subiliaci lymph node was obtained from the flank. The lymph nodes were excised during fabrication of the carcass after carcass chilling. Chuck and flank lymph nodes were collected from cull cows and bulls or fed cattle in sample sets that contained 95 of each type of lymph node. Sample sets were collected three times each from cull cows and bulls and fed cattle so that 570 lymph nodes (285 each of chuck or flank) from each cattle population were obtained, resulting in a total of 1,140 lymph nodes examined in this study. Because of the differences in carcass fabrication practices at the individual processing plants, flank lymph nodes were unavailable at one processing plant and the medial iliaci lymph node from the tenderloin was used instead. No effort was made to ensure that the flank and chuck lymph nodes came from the same animal. After harvest, the lymph nodes were placed on ice and shipped to the U.S. Meat Animal Research Center for analysis.

Sample processing. Upon arrival at the laboratory, excess fat and fascia was trimmed from each lymph node. Trimmed lymph nodes were surface sterilized by immersion for 3 s in a boiling water bath and then cut into multiple pieces with a sterile scalpel. The lymph node pieces were placed into a filtered stomacher bag (Nasco, Fort Atkinson, Wis.), weighed, and pulverized with a rubber mallet.

Enumeration. *Salmonella* were enumerated from lymph node samples using hydrophobic grid membrane filtration (HGMF) following previously described methods *(8).* Buffered peptone water (20 ml) was added to each sample bag and mixed for 60 s with a laboratory blender (BagMixer 400VW, Interscience Laboratories Inc., Weymouth, Mass.) at medium speed (seven strokes per second). A 250-μl aliquot of the resulting suspension was removed and place into a 15-ml conical tube containing 5 ml of phosphate-buffered saline (PBS) plus Tween 80 (Sigma, St. Louis, Mo.). The tubes were mixed by inversion and then allowed to sit at room temperature for 5 min to allow the meat and fat debris to settle. HGMF analysis was performed by applying 5 ml of the sample to an IsoGrid membrane (Neogen, Lansing, Mich.) and then filtering the sample using a Spread Filter apparatus (FiltaFlex Ltd., Almonte, Ontario, Canada). The membranes were transferred to xylose lysine desoxycholate (XLD) agar medium (Remel, St. Louis, Mo.) with 4.6 ml/liter Tergitol, 15 mg/liter novobiocin, and 5 mg/liter cefesulodin (XLDtnc; Sigma) and incubated at 37°C for 18 to 20 h. After incubation, colonies were counted, and the identity of suspect colonies was confirmed by PCR assay *(14, 17, 22).* The limit of detection in the enumeration assay was approximately 4 CFU/ml (1 CFU/0.25 ml analyzed by HGMF). *Salmonella* levels were calculated by determining the CFU per milliliter, converting to CFU per sample (given 20 ml per sample), and dividing by the recorded gram weight of the lymph node; final results were reported as CFU per gram of meat.

Sample processing for prevalence. After removing the 250 μl of lymph node sample for enumeration, the remaining sample was enriched with 80 ml of tryptic soy broth (Becton Dickinson, Sparks, Md.) and incubated at 25°C for 2 h and then 42°C for 6 h and held at 4°C overnight. After incubation, 1 ml from each enrichment culture was subjected to anti-*Salmonella* immunomagnetic separation (IMS). Each 1-ml aliquot received 20 μl of anti-*Salmonella* beads (Invitrogen, Carlsbad, Calif.), which were then extracted from the enrichment samples and washed twice in PBS–Tween 20 (Sigma) with an automated magnetic particle processor (KingFisher 96, Thermo Fisher Scientific, Inc. Waltham, Mass.). The beads were transferred to Rappaport Vassiliadis soya (RVS; Remel) broth and incubated at 42°C overnight. *Salmonella* present in these samples was detected by swabbing the RVS enrichment culture onto (i) Hektoen enteric agar (Becton Dickinson) containing novobiocin (5 mg/liter) and (ii) brilliant green medium with sulfadiazine (Becton Dickinson). All plates were incubated at 37°C for 18 to 20 h. After incubation, up to two suspect colonies were picked for confirmation. Suspect *Salmonella* isolates were confirmed by PCR assay using primers for the *Salmonella*-specific portion of the *invA* gene *(17, 22).*

Identification and characterization of multidrug-resistant *Salmonella.* *Salmonella* isolates were stamped in 96-well block format with a Boekel Microplate Replicator onto four tryptic soy agar (TSA) plates (150 mm) containing no antibiotics, ampicillin (32 mg/liter), tetracycline (32 mg/liter), or kanamycin (64 mg/liter). These plates were incubated at 37°C for 18 to 20 h. Isolates demonstrating resistance were streaked for isolation onto TSA with the appropriate antibiotic and were again incubated at 37°C for 18 to 20 h. Those yielding a pure culture on TSA were streaked for isolation on XLDtnc agar and incubated at 37°C for 18 to 20 h. Isolates demonstrating typical *Salmonella* colony morphology on XLDtnc (black colonies with a clear pink outer ring) were streaked for isolation onto TSA and incubated as described above. The resulting pure cultures were used for antibiotic sensitivity analysis and serological identification. Antibiotic sensitivity testing was performed using the Sensititre Broth Microdilution System (TREK Diagnostic Systems, Toledo, Ohio) and CMV1AGNF test plates, according to the manufacturer's instructions. The plates determine sensitivity to 15 antibiotics: amikacin, ampicillin, amoxicillin–clavulanic acid, ceftiofur, cefoxitin, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim-sulfamethoxazole. *Salmonella* isolates were serogrouped with the Welcollex Colour Serogrouping Kit (Remel) and further serotyped using O-factor and H-factor antisera (Remel) according to the manufacturer's instructions.

Statistical analysis. The mean and standard deviation (±SD) for the number of lymph nodes found to be contaminated with *Salmonella* were determined. Comparisons of the mean prevalence values for *Salmonella* in lymph nodes from cows and bulls or fed cattle were analyzed with Prism 4 GraphPad software (www.graphpad.com) using a two-tailed unpaired *t* test with Welch's correction for data with unequal variances ($P < 0.05$). The *P* values and 95% confidence intervals (95% CIs) of the differences between the population means are reported.

RESULTS AND DISCUSSION

Salmonella prevalence in the chuck and flank lymph node samples was low, with an overall prevalence of 1.6% (95% CI, 0.85 to 2.3%). Although lymph nodes from cull cattle had an overall higher prevalence of *Salmonella* than those from fed cattle ($P = 0.0175$, Table 1), the large variance in *Salmonella* contamination observed between sampling periods diminishes the significance of this difference. Flank nodes from cull cows had the highest individual prevalence at 3.86% (±4.25%), and lymph nodes from the chuck region of fed cattle had the lowest prevalence at

TABLE 1. *Observed prevalence of* Salmonella *in lymph nodes from chuck and flank trimmings*

Animal type	Chuck		Flank		Overall	
	No. of positive samples[a]	Mean (SD) prevalence (%)	No. of positive samples	Mean (SD) prevalence (%)	No. of positive samples	Mean (SD) prevalence (%)
Cull cattle	3/285	1.05 (1.82)	11/285	3.86 (4.25)	14/570	2.46 (3.31)
Fed cattle	1/285	0.35 (0.61)	3/285	1.05 (1.05)	4/570	0.70 (0.86)
$P > F$[b]		0.3166		0.0306		0.0175

[a] Number of samples that were positive for *Salmonella*/total number of samples.

[b] Comparisons of the mean prevalence values for *Salmonella* in lymph nodes from cull cow and bulls or fed cattle were analyzed using a two-tailed unpaired *t* test with Welch's correction for data with unequal variances.

0.35% (±0.61%). One flank node from a cull cow contained *Salmonella* at approximately 5.8 CFU/g. Although higher prevalences of *Salmonella* in bovine lymph nodes have been reported, in many of those studies lymph nodes that normally would not have been used in ground beef production were evaluated.

Mesenteric lymph nodes in close proximity to the intestinal tract commonly harbor enteric pathogens. Systemic translocation of multiple *Salmonella* isolates from the distal ileum can occur via the lymphatic system *(15, 16).* Samuel et al. *(19)* reported that 61 (72%) of 85 cattle had *Salmonella* in either the jejunal or cecal lymph nodes, and 29 of those cattle had at least one mesenteric lymph node that harbored *Salmonella* at more than 5,000 CFU/g. Few studies have been conducted on *Salmonella* in lymph nodes that could end up in ground beef production, such as those nodes found in the chuck or flank regions. Lymph nodes from the chuck and round regions previously had been found to harbor several bacterial species, although *Salmonella* was not one of those species *(11).* Lepovetsky et al. *(11)* investigated the potential causes of deep muscle spoilage and concluded that under inadequate refrigeration conditions, bacteria present in the lymph nodes of the chuck and round regions could cause deep spoilage in carcasses. Extrapolating from this finding, these lymph nodes could be a source of spoilage bacteria when incorporated into ground beef. If *Salmonella* had been present, as in the present study, it would have been a food safety concern. *Salmonella* prevalence has been reported as 2.2% on fresh beef cuts, with a range of 0 to 9.6% depending on the type of cut *(21).* In the present study, the prevalence of *Salmonella* within lymph nodes was approximately the same as that on the surface tissue of intact beef cuts.

TABLE 2. *Characterization of* Salmonella *isolates*

Animal type	*Salmonella enterica* subtype	Antibiotic resistance[a]	No. of lymph nodes
Cull cattle	Anatum	NR	1
	Blockley	NR	2
	Cerro	NR	5
	Montevideo	(Ap)(G)	1
	Muenster	NR	1
	Newport	AmApFT(Ax)CKSSuTe	1
	Thompson	NR	1
	Typhimurium	(Am)ApCSSuTe	1
	Typhimurium	(Am)ApGKSSuTeSxt	1
Fed cattle	Cerro	NR	1
	Montevideo	NR	1
	Senftenberg[b]	NR	1
	Typhimurium	K	1

[a] NR, not resistant. Antibiotics: Am, amoxicillin–clavulanic acid; Ap, ampicillin; F, cefoxitin; T, ceftiofur; Ax, ceftriaxone; C, chloramphenicol; G, gentamycin; K, kanamycin; S, streptomycin; Su, sulfisoxazole; Te, tetracycline; Sxt, sulfamethoxazole-trimethoprim. Parentheses indicate intermediate resistance (i.e., the MIC was increased but was below the resistance breakpoint).

[b] Presumptive serotype. Loss of viability precluded H typing of isolate. O typing revealed the isolate to be O1,3,19. *Salmonella* Senftenberg strains are common cattle isolates of this O type.

Of the 18 *Salmonella*-positive lymph node samples, 3 contained multidrug-resistant *Salmonella* (Table 2). All three of these samples were from lymph nodes removed from the carcasses of cull cattle: two contained *Salmonella* Typhimurium isolates that were resistant to six and eight antibiotics, and one contained *Salmonella* Newport, which was resistant to 10 antibiotics. A *Salmonella* Montevideo isolate with intermediate resistance (the MIC was increased but was below the resistance breakpoint) to ampicillin and gentamycin was isolated from a cull animal lymph node sample. Only one fed cattle lymph node harbored antibiotic-resistant *Salmonella.* A *Salmonella* Typhimurium isolate, resistant only to kanamycin, was recovered from a flank lymph node obtained from a fed beef processing plant.

Several studies have revealed that antibiotic-resistant *Salmonella* can be isolated from cattle *(2, 5, 9, 10).* Of the 102 *Salmonella* strains isolated from beef cattle at one feedlot, 97% were resistant to at least one antibiotic, and 21% were resistant to two or more antibiotics *(10).* In another study, 40% of the *Salmonella*-positive hide and carcass samples harbored only isolates that were susceptible to all 13 antibiotics tested, but the remaining 60% had *Salmonella* isolates that were resistant to one or more antibiotics *(2).* As in the current study, feedlot cattle have been reported to have lower prevalences of multidrug-resistant *Salmonella* than that found in nonfeedlot cattle *(5).* All of the *Salmonella* serotypes identified in this study have been associated previously with cattle *(9).*

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REFERENCES

1. Arthur, T. M., J. M. Bosilevac, D. M. Brichta-Harhay, N. Kalchayanand, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2007. Effects of a minimal hide wash cabinet on the levels and prevalence of *Escherichia coli* O157:H7 and *Salmonella* on the hides of beef cattle at slaughter. *J. Food Prot.* 70:1076–1079.
2. Bacon, R. T., J. N. Sofos, K. E. Belk, D. R. Hyatt, and G. C. Smith. 2002. Prevalence and antibiotic susceptibility of *Salmonella* isolated from beef animal hides and carcasses. *J. Food Prot.* 65:284–290.
3. Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of Shiga toxin–producing *Escherichia coli,* including O157:H7 and non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *J. Food Prot.* 66:1978–1986.
4. Barkocy-Gallagher, G. A., T. M. Arthur, G. R. Siragusa, J. E. Keen, R. O. Elder, W. W. Laegreid, and M. Koohmaraie. 2001. Genotypic analyses of *Escherichia coli* O157:H7 and O157 nonmotile isolates recovered from beef cattle and carcasses at processing plants in the midwestern states of the United States. *Appl. Environ. Microbiol.* 67:3810–3818.
5. Beach, J. C., E. A. Murano, and G. R. Acuff. 2002. Serotyping and antibiotic resistance profiling of *Salmonella* in feedlot and nonfeedlot beef cattle. *J. Food Prot.* 65:1694–1699.
6. Bosilevac, J. M., T. M. Arthur, T. L. Wheeler, S. D. Shackelford, M. Rossman, J. O. Reagan, and M. Koohmaraie. 2004. Prevalence of *Escherichia coli* O157 and levels of aerobic bacteria and *Enterobacteriaceae* are reduced when hides are washed and treated with cetylpyridinium chloride at a commercial beef processing plant. *J. Food Prot.* 67:646–650.
7. Bosilevac, J. M., X. Nou, M. S. Osborn, D. M. Allen, and M. Koohmaraie. 2005. Development and evaluation of an on-line hide decontamination procedure for use in a commercial beef processing plant. *J. Food Prot.* 68:265–272.
8. Brichta-Harhay, D. M., T. M. Arthur, J. M. Bosilevac, M. N. Guerini, N. Kalchayanand, and M. Koohmaraie. 2006. Enumeration of *Salmonella* and *Escherichia coli* O157:H7 in ground beef, cattle carcass, hide and fecal samples using direct plating methods. *J. Appl. Microbiol.* 103:1657–1668.
9. Centers for Disease Control and Prevention. 2005. *Salmonella* surveillance: annual summary, 2004. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta.
10. Fluckey, W. M., W. G. Loneragan, R. Warner, and M. M. Brashears. 2007. Antimicrobial drug resistance of *Salmonella* and *Escherichia coli* isolates from cattle feces, hides, and carcasses. *J. Food Prot.* 70:551–556.
11. Lepovetsky, B. C., H. H. Weiser, and F. E. Deatherage. 1953. A microbiological study of lymph nodes, bone marrow and muscle tissue obtained from slaughtered cattle. *J. Appl. Microbiol.* 1:57–59.
12. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe. 1999. Food-related illness and death in the United States. *Emerg. Infect. Dis.* 5:607–625.
13. Nou, X., M. Rivera-Betancourt, J. M. Bosilevac, T. L. Wheeler, S. D. Shackelford, B. L. Gwartney, J. O. Reagan, and M. Koohmaraie. 2003. Effect of chemical dehairing on the prevalence of *Escherichia coli* O157:H7 and the levels of aerobic bacteria and *Enterobacteriaceae* on carcasses in a commercial beef processing plant. *J. Food Prot.* 66:2005–2009.
14. Nucera, D. M., C. W. Maddox, P. Hoien-Dalen, and R. M. Weigel. 2006. Comparison of API 20E and *invA* PCR for identification of *Salmonella enterica* isolates from swine production units. *J. Clin. Microbiol.* 44:3388–3390.
15. Paulin, S. M., P. R. Watson, A. R. Benmore, M. P. Stevens, P. W. Jones, B. Villarreal-Ramos, and T. S. Wallis. 2002. Analysis of *Salmonella enterica* serotype-host specificity in calves: avirulence of *S. enterica* serotype Gallinarum correlates with bacterial dissemination from mesenteric lymph nodes and persistence in vivo. *Infect. Immun.* 70:6788–6797.
16. Pullinger, G. D., S. M. Paulin, B. Charleston, P. R. Watson, A. J. Bowen, F. Dziva, E. Morgan, B. Villarreal-Ramos, T. S. Wallis, and M. P. Stevens. 2007. Systemic translocation of *Salmonella enterica* serovar Dublin in cattle occurs predominantly via efferent lymphatics in a cell-free niche and requires type III secretion system 1 (T3SS-1) but not T3SS-2. *Infect. Immun.* 75:5191–5199.
17. Rahn, K., S. A. DeGrandis, R. C. Clarke, S. A. McEwen, J. E. Galan, C. Ginocchio, R. Curtiss, and C. L. Gyles. 1992. Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella. Mol. Cell. Probes* 6:271–279.
18. Samuel, J. L., D. A. O'Boyle, W. J. Mathers, and A. J. Frost. 1979. Isolation of *Salmonella* from mesenteric lymph nodes of healthy cattle at slaughter. *Res. Vet. Sci.* 28:238–241.
19. Samuel, J. L., D. A. O'Boyle, W. J. Mathers, and A. J. Frost. 1980. Distribution of *Salmonella* in the carcasses of normal cattle at slaughter. *Res. Vet. Sci.* 28:368–372.
20. Sofos, J. N., S. L. Kochevar, G. R. Bellinger, D. R. Buege, D. D. Hancock, S. C. Ingham, J. B. Morgan, J. O. Reagan, and G. C. Smith. 1999. Sources and extent of microbiological contamination of beef carcasses in seven United States slaughtering plants. *J. Food Prot.* 62:140–145.
21. Stopforth, J. D., M. Lopes, J. E. Shultz, R. R. Miksch, and M. Samadpour. 2006. Microbiological status of fresh beef cuts. *J. Food Prot.* 69:1456–1459.
22. Wang, R. F., W. W. Cao, and C. E. Cerniglia. 1997. A universal protocol for PCR detection of 13 species of foodborne pathogens in foods. *J. Appl. Microbiol.* 83:727–736.