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Cross-sectional Study Examining *Salmonella enterica* Carriage in Subiliac Lymph Nodes of Cull and Feedlot Cattle at Harvest

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Abstract

Bovine peripheral lymph nodes (LNs), including subiliac LNs, have been identified as a potential source of human exposure to *Salmonella enterica*, when adipose trim containing these nodes is incorporated into ground beef. In order to gain a better understanding of the burden of *S. enterica* in peripheral LNs of feedlot and cull cattle, a cross-sectional study was undertaken in which 3327 subiliac LNs were collected from cattle at harvest in seven plants, located in three geographically distinct regions of the United States. Samples were collected in three seasons: Fall 2010, Winter/Spring 2011, and Summer/Fall 2011. A convenience sample of 76 LNs per day, 2 days per season (approximately 1 month apart), was collected per plant, from carcasses held in the cooler for no less than 24 h. Every 10th carcass half on a rail was sampled, in an attempt to avoid oversampling any single cohort of cattle. Median point estimates of *S. enterica* contamination were generally low (1.3%); however, median *Salmonella* prevalence was found to be greater in subiliac LNs of feedlot cattle (11.8%) compared to those of cull cattle (0.65%). Enumeration analysis of a subset of 618 feedlot cattle LNs showed that 67% of those harboring *S. enterica* (97 of 144) did so at concentrations ranging from <0.1 to 1.8 $\log_{10}$ CFU/g, while 33% carried a higher burden of *S. enterica*, with levels ranging from 1.9 to >3.8 $\log_{10}$ CFU/g. Serotyping of *S. enterica* isolated identified 24 serotypes, with the majority being Montevideo (44.0%) and Anatum (24.8%). Antimicrobial susceptibility phenotypes were determined for all isolates, and the majority (86.1%) were pansusceptible; however, multidrug-resistant isolates (8.3%) were also occasionally observed. As *Salmonella* contained within LNs are protected from carcass interventions, research is needed to define opportunities for mitigating the risk of *Salmonella* contamination in LNs of apparently healthy cattle.

Introduction

Nontyphoidal *Salmonella enterica* is a significant cause of morbidity and mortality in the United States and is estimated to cause over 1 million illnesses each year (Mead *et al.*, 1999; Guo *et al.*, 2011; Scallan *et al.*, 2011). The vast majority of these cases are foodborne and while produce, poultry, and eggs account for most illnesses, beef has been identified as a vehicle of exposure, associated with sporadic cases and outbreaks (Guo *et al.*, 2011). *S. enterica* (hereafter referred to as *Salmonella*) has been frequently recovered from the hides and feces of healthy cattle (Barkocy-Gallagher *et al.*, 2003; Loneragan and Brashears, 2005; Brichta-Harhay *et al.*, 2008; Kunze *et al.*, 2008; Loneragan *et al.*, 2012), and it is theorized that animal carriage of *Salmonella* ultimately contributes to ground beef contamination (Bosilevac *et al.*, 2009).

During harvest, hides of cattle are likely the primary source of *Salmonella* contamination of carcass surfaces (Barkocy-Gallagher *et al.*, 2001; Barkocy-Gallagher *et al.*, 2003; Arthur *et al.*, 2008; Brichta-Harhay *et al.*, 2008). Accordingly, substantial effort is afforded to preventing contamination of carcasses and removal of contamination through strategies

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outlined in plant Hazard Analysis Critical Control Point plans. These strategies appear quite effective, as *Salmonella* prevalence after antimicrobial intervention application is typically undetectable or less than 1% (Barkocy-Gallagher *et al.*, 2003; Rivera-Betancourt *et al.*, 2004; Brichta-Harhay *et al.*, 2008). However, despite successful control of surface contamination, it is still possible for *Salmonella* to be recovered from ground beef. In a study of commercial ground beef from seven regions of the United States ($n = 4136$ samples collected over 2 years), *Salmonella* was recovered from 4.2% of ground beef samples (Bosilevac *et al.*, 2009). Similarly, government testing of ground beef indicates that *Salmonella* contamination averages around 2.1%, and that little improvement in contamination has been achieved over the past decade (FSIS, 2011), even while the prevalence of *Escherichia coli* O157:H7 in ground beef has declined more than 70% (from 0.80% in 2001 to 0.23% in 2010 [FSIS, 2012]).

Research suggests that pathogen contamination of ground beef also can occur by means of contaminated lymph nodes (LNs) (Arthur *et al.*, 2008). Cattle possess many LNs located within fatty tissues that are frequently incorporated into ground beef and thus have the potential to contaminate the final product. Contaminated LNs may explain the difference in *Salmonella* prevalence between postintervention carcasses or trim, and ground beef. When present in LNs, *Salmonella* are protected from chemical and thermal antimicrobial carcass interventions, and as a consequence sanitary harvest procedures may not address this potential source of contamination. Therefore, the objective of this cross-sectional study was to gain a better understanding of the burden of *S. enterica* in peripheral LNs of harvest-ready cattle, by examining the point prevalence, serotypes, and antimicrobial susceptibility phenotypes present in subiliac LNs of cull and fed cattle at harvest, in three regions of the United States.

Materials and Methods

Sample collection

Subiliac LNs were collected from carcasses of cull (cows/bulls and dairy) and feedlot (steers/heifers) cattle that had passed federal inspection at commercial abattoirs. Samples were collected during three time periods including September, October, and November 2010 (Fall 2010), February and March 2011 (Winter/Spring 2011), and July, August, and September 2011 (Summer/Fall 2011). A convenience sample of seven commercial processing plants consisting of three plants that primarily harvest feedlot cattle and four that primarily harvest cull cows was initially enrolled. An eighth plant, harvesting primarily feedlot cattle, contributed two sets of samples during the Summer/Fall 2011 sample period. Participating abattoirs were located in regions 2, 3, and 5 of the microbiological monitoring regions defined by the Beef Industry Food Safety Council (Fig. 1). A convenience sample of 76 LNs per day, 2 days per season (approximately 1 month apart), was collected per plant, from postintervention carcasses that had been held in the cooler for no less than 24 h. Approximately every 10th carcass half on a rail was sampled, resulting in sample collection from cattle harvested over 1–2 production days, in an attempt to avoid oversampling any single cohort of cattle. However, the possibility remains that multiple nodes from a single cohort reside in a given sample set.

FIG. 1. Map of the Beef Industry Food Safety Council microbiological monitoring regions from which fed and cull cattle lymph nodes were obtained.

Samples were shipped to Texas Tech University in Lubbock, Texas or the U. S. Meat Animal Research Center in Clay Center, Nebraska for processing.

LN sample processing and Salmonella *detection*

LNs were processed as previously described (Brichta-Harhay *et al.*, 2012). Briefly, surrounding fat and fascia were trimmed from LN samples, which were weighed, surface sterilized by submersion in a boiling water bath, placed into individual filtered sample bags (Nasco, Atlanta, GA), pulverized using a rubber mallet, and then enriched in 80 mL of tryptic soy broth (Becton Dickinson, Sparks, MD) by incubating at 25°C for 2 h and then 42°C for 12 h. Enrichments were subjected to immunomagnetic separation using anti-*Salmonella* beads (Dynabeads; Invitrogen, Carlsbad, CA). Recovered beads were transferred to 3 mL of Rappaport-Vassiliadis (Remel, St. Louis, MO) broth, incubated at 42°C for 18–20 h, then streaked onto xylose lysine desoxycholate (XLD; Remel, St. Louis, MO) and brilliant green sulfa (Becton Dickinson, Franklin Lakes, NJ) agar plates prior to incubation at 37°C for 18–20 h.

Presumptive *Salmonella* isolates were confirmed using *invA* polymerase chain reaction (Rahn et al., 1992; Nucera *et al.*, 2006). Isolates were subjected to molecular serotyping methods (Herrera-León *et al.*, 2004). Resulting phenotypes were further confirmed by traditional slide agglutination (O typing) and tube agglutination (flagellar H typing) methods, using commercial antisera (Difco, BD Diagnostic Systems, Sparks, MD) following manufacturer's guidelines.

Antimicrobial susceptibility testing

Susceptibility to 15 antimicrobial agents was determined using broth microdilution (Sensititre CMV1AGNF test plates; TREK Diagnostic Systems, Inc., Cleveland, OH) according to manufacturer's guidelines. Isolates were classified as susceptible, intermediate, or resistant for each agent using breakpoints established by the Clinical and Laboratory Standards Institute (CLSI, 2010). Isolates resistant to two or more classes of antimicrobials, as defined by the National Antimicrobial Resistance Monitoring System (FDA, 2011), were considered multi-drug resistant (MDR).

Salmonella *enumeration*

Salmonella prevalence values observed in the Fall 2010 sample period for feedlot cattle LNs indicated that enumeration analysis of this population could yield data on levels of *Salmonella* present in contaminated tissues. Conversely, enumeration of cull cattle LNs was not attempted because low prevalence suggested the analysis would yield little data. For enumeration, all feedlot cattle LNs collected in the Summer/Fall 2011 sample period ($n = 618$) were processed as described above and immediately following homogenization, 1 mL of tryptic soy broth/LN homogenate was plated onto Petrifilm™ Enterobacteriaceae Count Plates (EB; 3M Microbiology, St. Paul, MN) in quadruplicate, and incubated at 37°C for 22–26 h. Petrifilm plates were then held at 4°C until presumptive culture results were obtained. Colonies were counted and 10–100% (depending on number of colonies present) were streaked to XLD for further confirmation. Colonies indicative of *Salmonella* on XLD plates were counted and the proportion of *Salmonella*-positive colonies were applied to the EB Petrifilm counts, in order to estimate the *Salmonella* count for each enumerated sample. LNs observed to harbor *Salmonella*, but at concentrations below the limit of detection (~1 CFU/g) were designated in the <0.1 $\log_{10}$ CFU/g category.

Statistical analysis

Salmonella point prevalence was calculated for each set of LNs collected (n = approximately 76 per set) in a given processing day ($n = 44$ sample sets). Observed prevalence values were grouped by outcome and the frequency distribution was depicted as a histogram (Fig. 2). Median and mean point prevalence values were determined based on animal type, season, and region, and univariate analyses of results were performed using a one-way analysis of variance and Bonferroni's multiple comparison post-test, the Kruskal-Wallis test for nonparametric data and Dunn's multiple comparison post-test, or the Mann–Whitney t-test for nonparametric data, as indicated in the footnotes of Table 1. Enumeration data also were plotted as total estimated CFU/LN versus LN weight in grams (Fig. 3), and the geometric mean $\log_{10}$ CFU/g for enumerable samples (114 of 618) was determined. Comparisons of prevalence estimates and construction of data plots were performed using Prism 5.0d, GraphPad Software, Inc. (www.graphpad.com, San Diego, CA) and p values < 0.05 were considered significantly different.

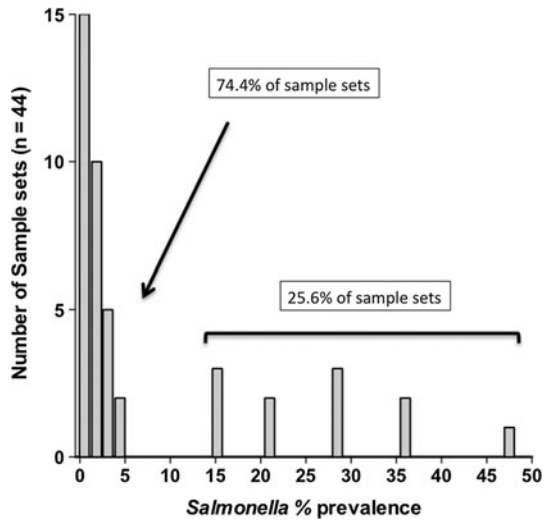

FIG. 2. Histogram of *Salmonella* prevalence outcomes for the 44 lymph node (LN) sample sets (n = approximately 76 LN per set) collected in this study.

Results

Salmonella *point prevalence and enumeration*

In this cross-sectional study, a total of 3327 subiliac LNs were collected from fed and cull cattle carcasses, in three regions of the United States, over 12 months (Table 1). Examination of the frequency distribution of observed prevalence values showed that the majority of sample sets (74.4%) had few-to-no *Salmonella* positives (Fig. 2). As a consequence, the median point estimate for *Salmonella* contamination was found to be 1.3%. However, some sample sets (25.6%) yielded considerably higher prevalence values. These sample sets skewed the resulting distribution such that arithmetic mean prevalence of *Salmonella* contamination was 7.5%. *Salmonella* point prevalence in feedlot cattle LNs was significantly greater than that observed in cull cattle ($p = 0.0006$) and appeared to be affected by region and season (Table 1), with levels significantly higher in region 3 as compared with region 5 ($p = 0.0198$), and in Summer/Fall 2011 as compared with Winter/Spring 2011 ($p = 0.0304$), for samples collected in region 3. Conversely, *Salmonella* prevalence in cull cattle LNs did not appear to be affected by region or season, as levels in this population tended to be low with a median overall point prevalence of 0.65%, although an exception to that trend was observed in region 5 in the Summer/Fall 2011 sample period (Table 1). It should be noted however, that further investigation revealed that the cull cows contributing to this outlier data point originated in region 3 but were transported to region 5 for harvest.

Enumeration analysis of 618 feedlot cattle LNs collected in Summer/Fall 2011 showed that 23.3% ($n = 144$) harbored *Salmonella* and that 18.4% ($n = 114$) contained levels detectable with the enumeration methods employed (limit of detection ~1 CFU/g). The geometric mean concentration of *Salmonella* for enumerable samples was 1.75 $\log_{10}$ CFU/g; however, as shown in Figure 3, a wide range of values were observed. While the majority of quantifiable nodes (58.8%; 67 of 114) contained *Salmonella* at concentrations ranging from 0.1 to 1.8 Log_{10} CFU/g, 41.2% (47 of 114) carried higher levels, ranging from 1.9 to 3.8 $\log_{10}$ CFU/g, or greater (Fig. 3).

Salmonella *serotypes and antimicrobial susceptibility phenotypes*

Twenty-four serotypes were identified, with the majority being either Montevideo (44.0%) or Anatum (24.8%; Table 2). Eighteen serotypes were identified among the 33 positive LNs from cull cattle, whereas 14 serotypes were observed among the 233 positive LNs from feedlot cattle. At least two colonies were serotyped for each positive LN, resulting in the isolation of multiple *Salmonella* serotypes from 3.8% ($n = 10$) of positive samples. Multiple serotypes were only observed in feedlot cattle LNs, and for prevalence estimates only one of the two serotypes was counted. Serotype combinations in these samples included Montevideo and Anatum ($n = 7$), Montevideo

Table 1. *Salmonella* Percent (%) Prevalence, and Standard Error (SE) in Subiliac Lymph Nodes (LNs) of Fed and Cull Cattle at Harvest, by Region and Season

	Cull cattle subiliac lymph nodes[a]				*Fed cattle subiliac lymph nodes*[a]			*Overall by season*
Sample period	*Region 2*	*Region 3*	*Region 5*	*All Cull*	*Region 3*	*Region 5*	*All Fed*	
Fall 2010								
Sample sets collected[b]	2	4	2	8	4	2	6	14
Number LNs tested	152	304	152	608	279	152	431	1039
Mean % (SE)	0.65 (0.65)	0.97 (0.62)	0	0.65 (0.35)	29.6 (1.5)	0	19.7[EF] (6.3)	8.8 (3.7)
Median %	0.65	0.65	0	0[D]	28.3	0	27.6	0.65
Minimum %	0	0	0	0	27.6	0	0	0
Maximum %	1.3	2.6	0	2.6	34.1	0	34.1	34.1
Winter/Spring 2011								
Sample sets collected[b]	2	4	2	8	4	2	6	14
Number LNs tested	151	305	152	608	305	147	452	1060
Mean % (SE)	3.3 (0.7)	0.32 (0.32)	0	0.98 (0.54)	2.3 (0.34)	0.7 (0.7)	1.8[E] (0.44)	1.3 (0.37)
Median %	3.3	0	0	0[D]	2.6	0.7	1.9	1.3
Minimum %	2.6	0	0	0	1.3	0	0	0
Maximum %	4.0	1.3	0	4	2.8	1.4	2.8	4.0
Summer/Fall 2011								
Sample sets collected[b]	2	4	2	8	6	2	8	16
Number LNs tested	152	306	152	610	466	152	618	1228
Mean % (SE)	1.3 (0)	0.65 (0.38)	12.5 (8.6)	3.8 (2.5)	24.7 (8.0)	13.2 (1.4)	21.4[F] (5.9)	12.0 (3.8)
Median %	1.3	0.65	12.5	1.3[D]	20.0	13.2	17.7	3.9
Minimum %	1.3	0	3.9	0	1.3	11.8	1.3	0
Maximum %	1.3	1.3	21.1	21.1	47.4	14.5	47.4	47.4
Overall by region or type								Lymph nodes overall
Sample sets collected[b]	6	12	6	24	14	6	20	44
Number LNs tested	455	915	456	1826	1050	451	1501	3327
Mean % (SE)	1.75 (0.56)	0.65 (0.25)	4.2 (3.4)	1.8 (0.88)	19.3 (4.4)	4.6 (2.7)	14.7 (3.5)	7.5 (1.9)
Median %	1.3[A]	0[A]	0[A]	0.65[G]	20.0[B]	0.7[C]	11.8[H]	1.3
Minimum %	0	0	0	0	1.3	0	0	0
Maximum %	4.0	2.6	21.1	21.1	47.4	14.5	47.4	47.4

[a]Common uppercase superscripts indicate values that are not significantly different ($p > 0.05$).
[b]Approximately 76 LNs collected per set, two sets collected per plant, approximately 1 month apart in each season.
[A]Median cull cattle prevalence by region was not significantly different ($p = 0.2356$) one-way analysis of variance (ANOVA) Kruskal–Wallis nonparametric test; Dunn's multiple comparison post-test.
[B/C]Median fed cattle prevalence by region was significantly different ($p = 0.0198$) Mann–Whitney nonparametric two-tailed *t*-test.
[D]Median cull cattle prevalence by season was not significantly different ($p = 0.3007$) one-way ANOVA Kruskal–Wallis nonparametric test; Dunn's multiple comparison post-test.
[E/F]Mean fed cattle prevalence by season was significantly different ($p = 0.0304$) one-way ANOVA and Bonferroni's post-test.
[G/H]Median lymph node prevalence overall for cull and fed cattle was significantly different ($p = 0.0006$) Mann–Whitney nonparametric two-tailed *t*-test.

and Infantis ($n = 1$), Montevideo and Muenchen ($n = 1$), and Anatum and Kentucky ($n = 1$).

The majority of *Salmonella* isolates (229 of 266) were susceptible to all antimicrobial agents tested. Multidrug resistance (MDR, defined as resistance to two or more antimicrobial drug classes; [FDA, 2011]) was observed in 8.3% ($n = 266$) of isolates (Table 3). Seventeen isolates (6.4%) exhibited the MDR-AmpC phenotype (co-resistance to at least ampicillin, chloramphenicol, streptomycin, sulfisoxazole, tetracycline, amoxicillin/clavulanic acid, ceftiofur, and ceftriaxone) (Gupta *et al.*, 2003; Kunze *et al.*, 2008). Serotypes demonstrating MDR-AmpC resistance included Reading ($n = 13$), Newport ($n = 3$), and Typhimurium ($n = 1$), although it should be noted that 11 of the 13 Reading isolated were from a single set of 76 LNs collected from feedlot cattle in the Summer/Fall 2011 sample period, and likely represent a cluster originating from a single lot of cattle at harvest (Table 3).

Discussion

Salmonella are versatile enteric pathogens noted for their ability to invade and survive within host lymphoid tissues

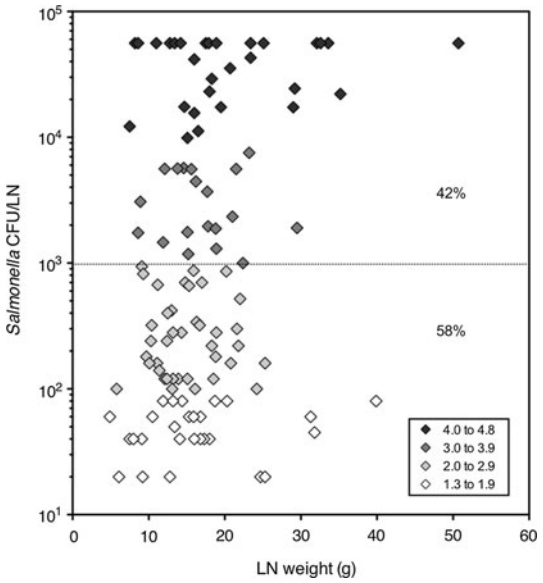

FIG. 3. Concentration of *Salmonella* in contaminated lymph nodes (LN) of feedlot cattle at harvest. Enumeration data were collected from 618 LN from carcasses of feedlot cattle in Summer/Fall 2011, using the methods described. Of these, 144 LN were positive for *Salmonella* contamination and 114 LN contained *Salmonella* at enumerable levels. Total estimated *Salmonella* CFU/LN was plotted versus LN weight (grams). Estimated total levels of *Salmonella* ($\log_{10}$ CFU/LN) are indicated by symbol color intensity (as depicted in the key), with white being the lowest (1.3–1.9 $\log_{10}$ CFU/LN) and dark gray being the highest (4.0–4.8 CFU/LN).

Table 2. *Salmonella* Serotypes Recovered from Subiliac Lymph Nodes (LNs) of Feedlot Cattle and Cull Cows

Serotype	*All LNs (n=266) overall %*	*Fed cattle LNs (n=233) relative %*	*Cull cattle LNs (n=33) relative %*
Montevideo	44.0	48.5	12.1
Anatum	24.8	27.5	6.1
Reading	4.9	5.2	3.0
Thompson[a]	3.8	3.9	3.0
Meleagridis	3.0	3.4	
Kentucky	3.0	1.7	12.1
C07 NT	2.3	2.6	
Mbandaka	2.3	1.3	9.1
Muenchen[a]	1.5	1.7	
Bredeney	1.1		9.1
Infantis	1.1	1.3	
Newport	1.1	0.9	3.0
Braenderup	0.8		6.1
Brandenburg	0.8		6.1
Cerro	0.8	0.9	
Dublin	0.8		6.1
Muenster	0.8	0.9	
Panama[a]	0.8		6.1
Saint Paul	0.8	0.4	3.0
Cubana	0.4		3.0
Give	0.4		3.0
Kiambu[a]	0.4		3.0
Typhimurium	0.4		3.0
Uganda	0.4		3.0

[a]Indicates predicted serotype—serotype data incomplete due to the presence of R H antigens that do not react with H antisera. Isolates designated as Thompson[a] may be Thompson (6,7,14: k: 1,5) or Ohio (6,7,14: b: l,w); isolates designated as Panama[a] may be Panama (1,9,12: l,v: 1,5) or Javiana (1,9,12: l,z_{28}: 1,5). CO7 NT – Nontypeable *Salmonella* that reacts with O-group 6,7 antisera, but H-antigens are nonreactive.

(Stevens *et al.*, 2009). Previous research has shown that cattle peripheral LNs can serve as a vehicle for *Salmonella* contamination, if fat trim containing these nodes is incorporated into ground beef (Arthur *et al.*, 2008; Samuel *et al.*, 1980). In this cross-sectional study, we also observed that *Salmonella* could be recovered from subiliac LNs, but additionally found that point estimates of prevalence in feedlot cattle populations appear to be greater than those in cull cattle populations, and that *Salmonella* harborage may be affected by season and region. While further study is needed to confirm the observed trends, these data nevertheless raise intriguing questions regarding the mechanism of *Salmonella* entry into bovine peripheral LNs, and the factors influencing this phenomenon. Possible explanations for the differences observed include diet effects and animal age, as well as management of cattle prior to harvest and differences in the prevalence of *Salmonella* in cattle environments.

Numerous studies have described a seasonal effect on *Salmonella* prevalence in cattle environments, with prevalence peaking in warmer months (summer and fall), and dipping in colder months (winter and spring) (APHIS, 2001; Barkocy-Gallagher *et al.*, 2003; Edrington *et al.*, 2004). Furthermore, mounting evidence from surveys of cattle feces, hides, and environments suggests a regional variation in *Salmonella* prevalence in North America, where the burden of *Salmonella* broadly increases across a southerly gradient. In a large study that included fecal samples of cattle housed in Canadian feedlots, *Salmonella* was recovered from 0.2% of cattle ready for harvest (Sorensen *et al.*, 2002). In the Midwest, Barkocy-Gallagher *et al.* (Barkocy-Gallagher *et al.*, 2003) reported peak fecal prevalence of 9.1% during the summer and fall months, while in Texas, *Salmonella* was recovered from 32.0% and 25.5% of fecal samples collected from healthy cattle housed in six feedlots and 22 dairies, respectively (Kunze *et al.*, 2008; Farrow *et al.*, 2009). The observed similarity in seasonal and regional prevalence between *Salmonella* in LNs and in cattle environments suggests the potential for an environmental component to the mechanism of how *Salmonella* gains entry to peripheral nodes. It is known that subiliac LNs receive afferent lymph from the skin of the abdominal wall, pelvis, prepuce, and hind limbs; thus, it is possible that *Salmonella* recovered from subiliac LNs may have entered via a transdermal route, through abrasions or biting insects. This idea has been suggested previously (Samuel *et al.*, 1980), and given that cattle hides are a common reservoir for *Salmonella* (Loneragan and Brasheears, 2005; Brichta-Harhay *et al.*, 2008; Kunze *et al.*, 2008), the observed correlation between *Salmonella* prevalence on cattle hides, in cattle environments, and in peripheral LNs is perhaps not surprising. The observed difference in prevalence between feedlot and cull cattle in region 3 was unexpected, however, and may reflect differences in hygiene or mitigation practices (i.e., *Salmonella* vaccine use or differences in pest management) because of a greater perceived risk of *Salmonella* as an animal health issue in dairy cattle populations.

Table 3. *Salmonella* Serotypes and Observed Antimicrobial Susceptibility Phenotypes Recovered from Subiliac Lymph Nodes of Feedlot Cattle and Cull Cows

Animal type	*Serotype*	*Antimicrobial resistance phenotype*[a]	*Number of lymph nodes*
Cull	Mbandaka	S	1
	Kentucky	S	1
	Dublin	Ap, C, K, G, Su	1
	Dublin	Ap, C, K, G, Su, Te	1
	Newport	Am, Ap, F, T, C, S, Su, Te	1
	Reading	Am, Ap, F, T, Ax, C, S, Su, Te	1
	Typhimurium	Am, Ap, F, T, Ax, C, S, Su, Te	1
Fed	Montevideo	Te	11
	Thompson	Te	1
	Montevideo	S	1
	Kentucky	Su, Te	1
	C O7 NT[b]	Su, TS	2
	Reading	Am, Ap, F, T, C, S, Su, Te	11[c]
	Newport	Am, Ap, F, T, Ax, C, S, Su, Te	2
	Reading	Am, Ap, F, T, Ax, C, S, Su, Te	1

[a]Am, amoxicillin-clavulanic acid; Ap, ampicillin; F, cefoxitin; K, Kanamycin; G, Gentamicin; T, ceftiofur; Ax, ceftriaxone; C, chloramphenicol; S, streptomycin; Su, sulfisoxazole; Te, tetracycline; TS, trimethoprim/sulphamethoxazole.

[b]CO7 NT, nontypeable *Salmonella* that reacts with O-group 6,7 antisera, but H-antigens nonreactive.

[c]Detected as a cluster of isolates originating from one set of 76 lymph nodes and thus may originate from a single lot of cattle at harvest.

Having confirmed that subiliac LNs may be a source of *Salmonella* contamination in ground beef, it was important to examine the serotypes and antimicrobial susceptibility profiles of *Salmonella* occupying this niche, because depending on host status, transmission vehicle, and inoculum level, certain *Salmonella* serotypes appear to be more relevant to causing human disease (Jones *et al.*, 2008). Results showed a diverse set of serotypes were isolated; however, two serotypes—Montevideo (44%) and Anatum (24.8%)—represented the majority of isolates (Table 2). These serotypes are frequently isolated from feces and hides of healthy feedlot cattle, especially in region 3 (Fluckey *et al.*, 2007; Kunze *et al.*, 2008), and as the majority of positive LN samples (79.7%) were collected from feedlot cattle in this region, their predominance is not unexpected. It is noteworthy, however, that Montevideo and Anatum are also the most commonly recovered *Salmonella* serotypes from ground beef in both federal testing programs (FSIS, 2011) and national surveys (Bosilevac *et al.*, 2009). Considering the enumeration data presented here, demonstrating that 33% of contaminated LNs tested (47 of 144) harbored *Salmonella* at levels in the range of 1.9 to >3.8 $\log_{10}$ CFU/g, it is tempting to suggest that these data identify the mechanism by which the majority of *Salmonella* may be entering ground beef. To our knowledge, this is the first report to document the range in *Salmonella* contamination present in cattle peripheral LNs.

Antimicrobial susceptibility phenotyping showed that the majority of *Salmonella* isolated (86%) were susceptible to all antimicrobials tested; however, MDR *Salmonella* (8.3%) were observed (Table 3). Notably, 15.2% of isolates from cull cattle LNs ($n=33$) were MDR while 7.3% of isolates from fed cattle ($n=233$) had MDR phenotypes. When considering *Salmonella* that are potentially more relevant to human disease, Typhimurium and Newport are two of the leading serotypes isolated in cases of human illness in the United States and have been associated with outbreaks attributed to contaminated ground beef (Gupta *et al.*, 2003). Conversely, serotypes Montevideo and Anatum have been implicated in fewer laboratory-confirmed human salmonellosis cases (CDC, 2011), especially from ground beef sources, and lack medical relevance in comparison. These observations highlight the need for investigation into the virulence factors, or adaptive mechanisms that may be associated with increased human illness among medically relevant serotypes. In this study, serotypes Typhimurium and Newport were observed in 6.1% and 0.9% of cull and fed cattle LNs, respectively (Table 3). Quantification of pathogen load in peripheral LNs containing these medically relevant serotypes will aid in modeling the potential quantitative risk imposed by the addition of contaminated LNs to ground beef.

Conclusions

The data presented show that subiliac LNs can be a significant source of *Salmonella,* if incorporated in ground beef, and that prevalence appears to be affected by season, region, and animal type. Furthermore, we show that contaminated nodes can carry substantial levels of *Salmonella* (1.9 to >3.8 $\log_{10}$ CFU/g). As LN harborage protects *Salmonella* from carcass interventions, research is needed to define opportunities for mitigating the risk of *Salmonella* contamination in LNs of apparently healthy cattle.

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Disclosure Statement

No competing financial interests exist.

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