frontiers in Animal Science

ORIGINAL RESEARCH
published: 21 March 2022
doi: 10.3389/fanim.2022.859800

Salmonella Carriage in Peripheral Lymph Nodes and Feces of Cattle at Slaughter Is Affected by Cattle Type, Region, and Season

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Salmonella is a significant food safety concern in commercial beef production, and some contamination is thought to occur by inclusion of *Salmonella*-infected peripheral lymph nodes (LN) in ground beef and through fecal contamination. Surveillance in processing plants assists packers in risk management of *Salmonella* by understanding seasonal trends and risks associated with different cattle types. Approximately 25 fecal samples and 20 LN were collected from animals representing each of five cattle types (cull beef cattle, cull dairy cows, conventional feedlot cattle, all-natural feedlot cattle raised without pharmaceuticals, and grass-finished cattle) and each of five climate regions (mixed-temperatures and dry, mixed-temperatures and humid, hot and humid, hot and dry, cold) during each of three seasons (summer, fall, winter) to better characterize *Salmonella* inputs into a commercial cattle processing facility. In total, 1,840 fecal samples and 1,550 LN samples were collected. Fecal samples and LN were cultured for *Salmonella*, and select isolates were serogrouped and screened for antimicrobial resistance. Conventional feedlot cattle had the highest LN *Salmonella* concentrations (1.17 log10 CFU/g LN) in this data set, while cull dairy cows had the highest fecal *Salmonella* concentrations (1.96 log10 CFU/g feces). Conventional feedlot cattle and cull dairy cows had the greatest *Salmonella* prevalence in both LN (32 and 18%, respectively) and feces (37 and 49%, respectively), while all-natural feedlot cattle had the lowest prevalence in the LN (3%) and feces (7%). As expected, *Salmonella* prevalence and concentration was lowest for all cattle types during winter compared to warmer seasons. When examined by climate region, a greater *Salmonella* prevalence in both feces and LN was observed in climate region 4 (hot-dry), than the other regions. Only 21 of 50 *Salmonella* isolates examined for antimicrobial susceptibility were identified as multidrug resistant (MDR); cull dairy cows were responsible for 48% of MDR isolates, cull beef cattle were responsible for 38%, and conventional feedlot, grass-fed, and all-natural feedlot cattle were each responsible for 4.8%. These results indicate that different production schemes, season, and climate region may influence which cattle are most likely to introduce *Salmonella* to the abattoir, allowing for greater risk awareness during the slaughter process.

Keywords: cattle, climate region, feces, lymph nodes, *Salmonella*, season

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Edited by:
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Specialty section:
This article was submitted to Animal Nutrition, a section of the journal Frontiers in Animal Science

Received: *21 January 2022*
Accepted: *23 February 2022*
Published: *21 March 2022*

Citation:
Wottlin LR, Edrington TS and Anderson RC (2022) Salmonella Carriage in Peripheral Lymph Nodes and Feces of Cattle at Slaughter Is Affected by Cattle Type, Region, and Season. Front. Anim. Sci. 3:859800. doi: 10.3389/fanim.2022.859800

INTRODUCTION

Despite decades of advancements in food safety, *Salmonella* is still occasionally found in retail beef. Peripheral lymph nodes (LN) harboring *Salmonella* are thought to be a primary source of ground beef contamination, as LN are sheltered within adipose or muscle tissue from post-harvest carcass sanitation procedures. Research has shown differences in prevalence of *Salmonella*-positive LN depending on season, region, and cattle source (Webb et al., 2017; Nickelson et al., 2019), with prevalence often increased in feedlot cattle compared to cull breeding animals, animals raised in southern latitudes compared to northern regions, and in warmer months compared to colder months. More recently, anecdotal observations from within the meat packing industry suggested that these trends may not always hold true. Regardless, research is scarce on the effects of other cattle types and climate regions on prevalence patterns.

Commercial beef processors that slaughter multiple cattle types from different regions within the U.S. are aware that differences in *Salmonella* burden coming into their plants may exist, and these patterns should be elucidated to the greatest extent possible. The results of a longitudinal study requested by a commercial beef processer are presented herein. Fecal samples and subiliac LN were collected for the culture of *Salmonella* at slaughter from a commercial cattle processing facility, representing cattle of different type, sourced from various climate regions of the southern United States, across seasons. Serogroup and antimicrobial resistance were also evaluated on a portion of the *Salmonella* isolates.

MATERIALS AND METHODS

Sample Collection

Samples were collected by plant personnel from a single commercial beef processing plant in the southwestern U.S. Collections were made with the goal of obtaining 25 fecal samples and 20 LN from animals representing each cattle type and climate region (detailed below) during each season of the year. Overall, 1,840 fecal samples and 1,550 subiliac LN were collected. Paired fecal samples and LN were not intentionally collected, however some samples may have been collected from the same animal.

Fecal samples (∼100 g) were collected from the rectum of individual cattle using a clean palpation sleeve, then placed into a sterile specimen cup for shipment. The subiliac LN, within the surrounding adipose tissue, were placed in individual sterile sample collection bags. Following collection, samples were shipped on ice overnight to the USDA-ARS laboratory in College Station, TX for bacterial culture as described below.

Cattle Type and Climate Regions

Five cattle types were examined: conventional feedlot cattle (raised with pharmaceuticals and technologies which increase feed efficiency, digestive health, and carcass quality); all-natural feedlot cattle (raised without antibiotics, exogenous growth-promoting hormones, or in-feed technologies such as ionophores and beta-agonists); grass-fed cattle (raised to market weight with varying amounts of pasture access); cull dairy cows (primiparous and multiparous); and cull beef cattle (cows and bulls). The host packing plant provided information on cattle source for this study. Five climate regions where cattle originated were represented in the sample collections (**Figure 1**; adapted from Building America climate map from the Office of Energy Efficiency and Renewable Energy, 2012): Region 1, mixed temperatures and dry; 2, mixed temperatures and humid; 3, hot and humid; 4, hot and dry; 5, cold. Cattle originated from feedlots, farms, and ranches within ∼970 km of the Texas panhandle. Samples were collected from these cattle types and regions in the summer, fall, and winter of 2019 but due to the development of the COVID-19 pandemic, were not collected in the spring of 2020.

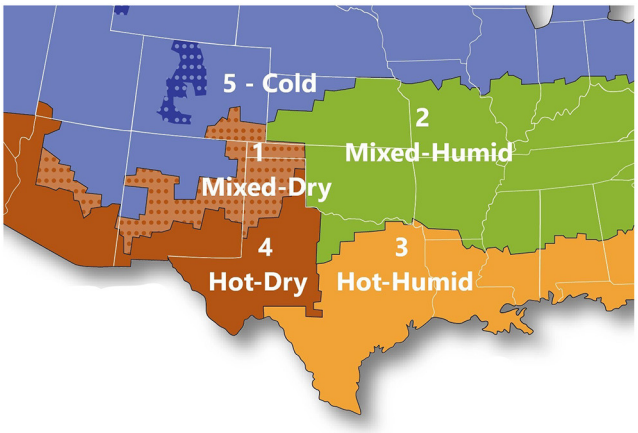

FIGURE 1 | Climate regions from which cattle originated. Adapted from (https://www.energy.gov/eere/buildings/building-america-climate-specific-guidance).

Salmonella Culture

Unless otherwise stated, all media and agar were sourced from Difco Laboratories (Detroit, MI), and reagents and antibiotics were obtained from Sigma Chemical Co. (St. Louis, MO). Fecal samples were analyzed as described previously (Brichta-Harhay et al., 2007). Briefly, 10 g of each fecal sample was enriched in 90 mL tetrathionate broth containing 1.8 mL of iodine solution for 24 h at 37°C. For enumeration of *Salmonella*, a 50 μL aliquot of the pre-incubation mixture was plated onto XLD using a spiral plater, incubated for 24 h at 37°C, and then an additional 24 h at room temperature. Following incubation of the feces-tetrathionate mixture, a 100 μL aliquot was transferred to 5 mL Rapport-Vassiliadis R10 (RV) broth and incubated an additional 24 h at 42°C. After this selective enrichment, samples were dual-plated onto brilliant green agar (BGA) with novobiocin (25 μg/mL) and BGA with tetracycline (30 μg/mL) to assist in

Abbreviations: LN, Lymph node; MDR, Multidrug-resistance.

the identification of isolates for multidrug resistance (MDR) testing. All plates were incubated for 24 h at 37°C, then up to three suspect colonies (pink with distinct round border) per plate were biochemically confirmed using lysine iron and triple sugar iron agars.

Lymph nodes were processed and cultured quantitatively and qualitatively for *Salmonella* as described by Brichta-Harhay et al. (2012). Lymph nodes were trimmed of excess fat and fascia, then surface sterilized by immersion in boiling water for 3–5 s. The surface-sterilized LN was placed in a filtered stomacher bag, weighed, and pulverized using a rubber mallet. Next, 80 mL of tryptic soy broth (TSB) was added and the mixture homogenized for 30 s using a laboratory stomacher. For enumeration, 1 mL of the homogenized mixture was applied to Petrifilm EB (3M Health Care, St. Paul, MN) in duplicate and incubated at 37°C overnight. Films with bacterial growth were transferred to XLD plates containing 10 μg/mL cefsoludin and 15 μg/mL novobiocin and incubated (24 h, 37°C). Black colonies were counted and converted to $\log_{10}$ CFU/g LN. For prevalence analysis, the original LN-TSB mixture was incubated at room temperature for 2 h, then for 12 h at 42°C. Next, 1 mL from each enrichment culture was subjected to anti-*Salmonella* immunomagnetic separation with 20 μL of anti-*Salmonella* beads (Invitrogen, Waltham, MA) and incubated with shaking at 800 rpm at room temperature for 15 min. The beads were extracted from the enrichment samples and washed twice in PBS-Tween 20, then were transferred to 3 mL of RV broth and incubated (24 h, 42°C). Finally, 100 μL of RV broth was plated each to BGA with sulfadiazine (80 μg/mL), and BGA with tetracycline (30 μg/mL), then incubated at 37°C overnight. Lastly, suspect colonies were biochemically confirmed using lysine iron and triple sugar iron agars.

Serogrouping and Antimicrobial Susceptibility Testing

Isolates which grew on BGA with tetracycline were re-streaked on tryptic soy agar with 5% sheep blood (Becton, Dickenson and Co., Sparks, MD) for serogrouping using slide agglutination with *Salmonella* antiserum and antimicrobial susceptibility testing. Serogroups were confirmed by traditional slide agglutination (O typing) methods, using commercial antisera (Becton, Dickinson and Co.) following the manufacturer's guidelines.

Susceptibility to 14 antimicrobial agents was determined by use of an automated broth microdilution method (Sensititre Gram Negative NARMS Plates, TREK Diagnostics Inc., Oakwood Village, OH) according to the manufacturer's recommendations. Minimum inhibitory concentrations (MICs) were determined using the breakpoints established by the Clinical and Laboratory Standards Institute (CLSI, 2017) or by National Antimicrobial Resistance Monitoring System (NARMS, 2019) when CLSI criteria were not established, in order to classify isolates as susceptible or resistant. Isolates that were resistant to three or more classes of antimicrobials were considered MDR.

Statistical Analysis

Data were compiled and organized using Excel (Microsoft Corp., Redmond, WA), and then were analyzed using JMP 15 (SAS Institute Inc., Cary, NC). Fecal samples that were below the limit of detection (∼10 CFU/g) during direct plating for enumeration but were positive for *Salmonella* after enrichment, were assigned a concentration of 1 $\log_{10}$ CFU/g feces (Brichta-Harhay et al., 2007). Similarly, lymph nodes that were below the limit of detection (∼1 CFU/g) but were positive following enrichment, were assigned a concentration of 0.1 $\log_{10}$ CFU/g lymph node.

To examine effects of season and cattle production type on *Salmonella* recovery in feces and peripheral LN, season and cattle type were included in the model as fixed effects, and the sample ID within cattle type was included as a random effect. To examine effect of climate region on *Salmonella* recovery in feces and peripheral LN, climate region was included as a fixed effect while cattle type was included as a random effect, due to imbalance of cattle type across regions. Quantitative concentration data were log-transformed prior to analysis to stabilize variances. Concentration data were analyzed using ANOVA, with Student's *t*-test for pairwise comparisons of treatment means when warranted. Prevalence data were analyzed using nominal logistic regression, with chi-square tests for significance. Significance was declared at $P \leq 0.05$.

RESULTS

A total of 1,550 subiliac LN and 1,840 fecal samples were collected across the five cattle types, in five climate regions, across three seasons. Overall, concentration ranged from 0.1 to 3.8 $\log_{10}$ CFU/g in LN, with 79% of positive samples having concentrations $< 1 \log_{10}$ CFU/g. In fecal samples, concentrations ranged from 1.0 to 6.2 $\log_{10}$ CFU/g, with 72% of positive samples' concentrations equal to 1.0 $\log_{10}$ CFU/g. There were 8 and 4.8% of samples quantitatively positive (detectable prior to enrichment) for *Salmonella* in feces and LN, respectively, and prevalence was 29.3 and 13.9% feces and LN, respectively.

Season and Cattle Type

Results of the *Salmonella* culture from the subiliac LN of cattle are presented in **Table 1** by cattle type, across all seasons and for each season. Across all seasons, conventional feedlot cattle had the greatest ($P < 0.01$) *Salmonella* concentration (1.17 $\log_{10}$ CFU/g LN), while grass-fed cattle had the least concentration (0.12 $\log_{10}$ CFU/g LN). Conventional feedlot cattle and cull dairy cows had a greater percentage of quantitatively positive LN and a greater prevalence rate compared to other cattle types ($P < 0.01$). Summer represented the season with the highest prevalence for each cattle type except cull dairy cows and conventional feedlot cattle, for which autumn was the season of highest prevalence. In all-natural feedlot cattle, autumn represented the time of lowest prevalence, but in all other cattle types, the lowest prevalence occurred through winter. During winter, there were no differences ($P > 0.70$) in concentration or prevalence between cattle types.

Results of the *Salmonella* recovery from fecal samples of different cattle types across season are presented in **Table 2** by cattle type, across all seasons and for each season. Across all seasons, cull dairy cows had the greatest ($P < 0.01$) *Salmonella* concentration (1.9 $\log_{10}$ CFU/g feces), compared to all other

TABLE 1 | Characterization of *Salmonella*-positive subiliac lymph nodes (LN) collected from different cattle types by and across three seasonal collections.

	Cattle type[a]					
Item[b]	**Cull beef**	**Cull dairy**	**Conventional feedlot**	**Grass-fed**	**All-natural feedlot**	***P*-value**
All seasons						
No. samples	370	549	183	288	160	
Concentration, log_{10}CFU/g LN	0.24	0.63	1.17	0.12	0.33	<0.01
Quantitatively positive, %	0.81	5.83	16.9	0.69	0.63	<0.01
Prevalence, %	7.0	17.9	31.7	8.7	3.1	<0.01
Summer						
No. samples	150	180	60	90	50	
Concentration, log_{10}CFU/g LN	0.26	0.66	1.7	0.13	0.49	<0.01
Quantitatively positive, %	1.33	6.11	33.3	2.20	2.00	<0.01
Prevalence, %	11.3	19.4	41.7	13.3	6.00	<0.01
Autumn						
No. samples	100	169	63	119	72	
Concentration, log_{10}CFU/g LN	0.10	0.61	0.74	0.10	0.10	0.16
Quantitatively positive, %	0	11.8	17.5	0	0	<0.01
Prevalence, %	7.00	34.9	49.2	10.1	1.39	<0.01
Winter						
No. samples	120	200	60	79	38	
Concentration, log_{10}CFU/g LN	0.54	0.79	0.10	0.10	0.10	0.93
Quantitatively positive, %	0.83	0.50	0	0	0	0.76
Prevalence, %	1.67	2.00	3.33	1.27	2.63	0.93

[a]*Cull beef, cull breeding beef cattle; Cull dairy, primi- and multiparous cull dairy cows; Conventional feedlot, feedlot cattle raised with conventional growth-promoting technologies; Grass-fed, cattle finished on grass; All-natural feedlot, feedlot cattle raised without antibiotics, exogenous hormones, or feed-through pharmaceuticals.*
[b]*Concentration data includes only* Salmonella*-positive samples.*

cattle types ($\leq$1.6), though that difference may not be biologically significant. Across all seasons, the prevalence rate in fecal samples was greater than that of the LN for each cattle type. The greatest ($P < 0.01$) percent quantitatively positive fecal samples and prevalence rate again occurred in cull dairy and conventional feedlot cattle, while all-natural feedlot cattle had the lowest. In autumn, cull dairy cows had the greatest ($P < 0.01$) *Salmonella* concentration (2.24 log_{10} CFU/g feces), compared to all other cattle types ($\leq$1.2 log_{10} CFU/g). Winter persisted as the season with the least qualitative prevalence in feces within each cattle type, but cull dairy cows had substantially greater ($P < 0.01$) prevalence rate (35.8%) during that season compared to other types ($\leq$10%).

Climate Region

Differences in *Salmonella* culture by climate region of origin for subiliac LN and fecal samples are presented in **Table 3**. Differences in *Salmonella* concentration among the LN and fecal samples across region are not likely of a biologically-significant magnitude, despite statistical significance, as all means were within 1 log_{10} CFU/g. Region 4 (hot-dry) presented the greatest prevalence of *Salmonella* in the LN, and region 2 (mixed temperature-humid) presented the lowest ($P < 0.05$). Region 4 also had the greatest *Salmonella* prevalence in fecal samples, while region 5 (cold) presented the lowest ($P < 0.01$).

Multi-Drug Resistance

Isolates from positive BGA_{tet} plates ($n = 50$) were serogrouped and subjected to antimicrobial susceptibility testing, and classified as MDR if resistance to three or more classes of antimicrobials was exhibited. Resistance patterns and source cattle types of MDR *Salmonella* isolates, by serogroup, are presented in **Table 4**. Twenty-one MDR isolates were identified, and only 4 isolates originated from LN (all of which were from cull dairy cows) while the rest were of fecal origin. Further, cull dairy cows were responsible for 48% (10/21) of all the MDR isolates, while cull beef cattle were responsible for 38% (8/21), and conventional feedlot, grass-fed, and all-natural feedlot cattle were each responsible for 4.8% (each 1/21). Serogroups C_1 and B represented the most frequent serogroups among MDR isolates (43 and 33%, respectively), though this may just reflect greater prevalence of these serogroups in general, as pansusceptible isolates were not serogrouped for comparison.

DISCUSSION

It is well-established that cattle may harbor *Salmonella* in the intestinal tract, thereby presenting the opportunity for fecal contamination of carcasses at slaughter, a significant food-safety risk (Koohmaraie et al., 2012; Muñoz-Vargas et al., 2018). Further, as *Salmonella* sequestered in the peripheral LN of cattle is protected from in-plant interventions, it may provide

TABLE 2 | Characterization of *Salmonella*-positive fecal samples collected from different cattle types by and across three seasonal collections.

	Cattle type[a]					
Item[b]	**Cull beef**	**Cull dairy**	**Conventional feedlot**	**Grass-fed**	**All-natural feedlot**	***P*-value**
All seasons						
No. samples	480	642	206	330	182	
Concentration, log_{10}CFU/g feces	1.38	1.96	1.40	1.60	1.00	<0.01
Quantitatively positive, %	1.88	17.6	5.83	3.64	0	<0.01
Prevalence, %	15.4	48.8	37.4	19.4	6.59	<0.01
Summer						
No. samples	160	222	73	100	60	
Concentration, log_{10}CFU/g feces	1.63	1.81	1.63	1.97	–	0.67
Quantitatively positive, %	3.13	16.7	21.9	9.00	0	<0.01
Prevalence, %	16.9	52.7	71.2	31.0	0	<0.01
Autumn						
No. samples	160	180	63	150	72	
Concentration, log_{10}CFU/g feces	1.15	2.24	1.05	1.21	1.00	<0.01
Quantitatively positive, %	1.25	26.7	1.59	1.33	0	<0.01
Prevalence, %	19.4	60.6	50.8	20.0	16.7	<0.01
Winter						
No. samples	160	240	70	80	50	
Concentration, log_{10}CFU/g feces	1.39	1.83	1.95	1.75	–	0.66
Quantitatively positive, %	1.25	12.1	1.43	1.25	0	<0.01
Prevalence, %	10.0	35.8	2.86	3.75	0	<0.01

[a]*Cull beef, cull breeding beef cattle; Cull dairy, primi- and multiparous cull dairy cows; Conventional feedlot, feedlot cattle raised with conventional growth-promoting technologies; Grass-fed, cattle finished on grass; All-natural feedlot, feedlot cattle raised without antibiotics, exogenous hormones, or feed-through pharmaceuticals.*
[b]*Concentration data includes only* Salmonella*-positive samples.*

TABLE 3 | Prevalence of *Salmonella* in subiliac lymph nodes and feces in cattle at harvest by climate region.

	Climate region[a]					
Item[b]	**1**	**2**	**3**	**4**	**5**	***P*-value**
Lymph nodes (LN)						
No. samples	501	478	219	182	170	–
Concentration, log_{10}CFU/g LN	0.94	0.84	0.38	0.38	0.13	<0.01
Quantitatively positive, %	6.98	3.77	3.34	4.39	1.18	0.52
Prevalence, %	15.6	9.41	17.2	19.8	11.2	0.04
Feces						
No. samples	615	572	210	213	230	–
Concentration, log_{10}CFU/g feces	0.59	0.52	0.38	0.75	0.18	<0.01
Quantitatively positive, %	8.6	9.6	4.0	12.7	1.3	0.03
Prevalence, %	35.1	27.4	30.5	36.6	14.8	<0.01

[a]*Climate region: 1, Mixed temperatures and dry; 2, Mixed temperatures and humid; 3, Hot and humid; 4, Hot and dry; 5, Cold and dry.*
[b]*Concentration data includes only* Salmonella*-positive samples.*

a source for potential ground beef contamination (Brichta-Harhay et al., 2012; Webb et al., 2017; Nickelson et al., 2019; Gutiérrez et al., 2020).

Cattle Type

Cattle types are not always clearly defined in study reports. For this study, conventional feedlot cattle were those raised with pharmaceuticals and technologies which increase feed efficiency, digestive health, and carcass quality (e.g., growth-promoting implants, antibiotics, ionophores), while all-natural feedlot cattle were raised in a similar environment but without exogenous pharmaceuticals. Grass-fed cattle were raised to market weight with varying amounts of access to pasture. Cull dairy cows included primiparous and multiparous cows removed from production for unrecorded reasons. Cull beef cattle included cows and bulls, removed from production for

TABLE 4 | Multi-drug resistant *Salmonella* isolates by serogroup isolated from subiliac lymph nodes ($n = 4$) and feces ($n = 17$) of cattle, by antimicrobial resistance pattern and source.

	Serogroup				
Resistance Pattern[a]	**B**	**C_1**	**C_2**	**D**	**E_1**
AMP AMC AXO CHL FOX STR TET TIO CIP NAL GEN SXT		1			
AMP AMC AXO CHL FOX STR TET TIO CIP NAL	3				
AMP AMC AXO CHL FOX STR TET TIO CIP	3				
AMP AMC AXO CHL FOX STR TET TIO	1				
AMP AMC AXO CHL FOX STR TET			1	1	
AMP CHL STR TET					2
CHL STR TET		8			1
Cattle type					
Cull beef cattle	7				1
Cull dairy cows		9		1	
Conventional feedlot cattle			1		
Grass-fed cattle					1
All-natural feedlot cattle					1

[a]*AMP, ampicillin; AMC, amoxicillin/clavulanic acid; AXO, ceftriaxone; CHL, chloramphenicol; FOX, cefoxitin; STR, streptomycin; TET, tetracycline; TIO, ceftiofur; CIP, ciprofloxacin; NAL, nalidixic acid; GEN, gentamicin; SXT, trimethoprim-sulfamethoxazole.*

unrecorded reasons. In the present study, conventional feedlot cattle presented the greatest prevalence of *Salmonella* in LN (32%) followed by cull dairy cattle (18%), while the other cattle types had substantially lower prevalence. Koohmaraie et al. (2012) also observed 18% prevalence rate in the cervical LN of 100 cull dairy cattle at harvest. Webb et al. (2017) reported a lower *Salmonella* prevalence of 7% in LN of conventional feedlot cattle and of 1.8% in LN from cull (mixed beef and dairy) cattle, and Brichta-Harhay et al. (2012) reported a mere 0.8% *Salmonella* prevalence in the LN of cull beef and dairy cattle. Alternatively, Gragg et al. (2013a) and Levent et al. (2019) each reported ∼75% prevalence rate in LN from fed cattle at slaughter. More recently, a study of 400 Mexican feedlot cattle reported a *Salmonella* prevalence of 9.7% on the peripheral LN (Gutiérrez et al., 2020). As it is posited that *Salmonella* primarily reaches the peripheral LN by escaping the gut, it is reasonable to assume that diet may have an effect on *Salmonella* prevalence within the LN, as it does in liver abscesses (Amachawadi and Nagaraja, 2015; Sanz-Fernandez et al., 2020). Further, cattle in feedlots and dairy systems are typically confined such that animal concentration per land area is greater, compared to pasture-based systems, which likely results in increased exposure to other animals, feces, and mud. Regardless, more research is needed to further investigate correlations between live animal behavior, diet and management, and *Salmonella* prevalence in the LN.

Research is limited regarding *Salmonella* culture from LN of all-natural feedlot cattle; however, previous research by the authors found relatively high *Salmonella* concentrations and prevalence in this cattle type (Edrington, unpublished data). Based on this previously-collected data and the fact that all-natural feedlot cattle typically receive a ration very similar to that of conventional feedlot cattle simply without antibiotics and other growth-promoting supplements, it was expected that similar *Salmonella* prevalence would be observed among the two types of feedlot cattle. Generally, the only difference between all-natural and conventional feedlot cattle is the use of antibiotics and growth-promoting compounds outlined above. Often, both types of cattle may be found within the same feedlot, managed and housed similarly with the above exceptions. The very low *Salmonella* concentration and prevalence observed in feces and LN from all-natural feedlot cattle and young grass-fed cattle in the present study is intriguing, and warrants further investigation. Continued research comparing these cattle types will help elucidate what factors, such as pen density and use of growth-promoting technologies, influence *Salmonella* burden in market cattle.

Research regarding *Salmonella* prevalence in feces varies widely across and within cattle types. Muñoz-Vargas et al. (2018) reported that periparturient dairy cows had increased fecal *Salmonella* shedding 1 wk prior and 1 wk following parturition (∼54%) compared to 3 wk prior to and 3 wk post-parturition (∼37%), evidencing that increased physiological stress may lead to increased *Salmonella* prevalence. Similarly, Edrington et al. (2004) reported an overall average of 39% *Salmonella* fecal prevalence rate in healthy lactating dairy cows, but prevalence varied widely across farm and season. Manishimwe et al. (2021) recently reported a fecal *Salmonella* prevalence rate of 18.8% among healthy dairy cows in Texas. The fecal samples in the present study were collected at time of harvest and a much greater prevalence rate (49%) in cull dairy cows was observed. Kunze et al. (2008) collected feces from fresh fecal pats at a feedlot and reported prevalence of 32%, which is similar to what was observed in the present study in conventional feedlot cattle. Dargatz et al. (2016) reported a fecal *Salmonella* prevalence rate of 9% in cattle in feedlots across 12 states which is substantially lower than what was observed in the present study (37%), but the following year Gragg et al. (2013a) reported a 94% fecal *Salmonella* prevalence rate in fed cattle at slaughter. In a similar trend to what was observed in the present study, Fegan et al. (2004) reported 4.5% fecal *Salmonella* prevalence in grass-fed cattle at slaughter, compared to 9% in grain-fed cattle. However, Barlow et al. (2015) reported 13% fecal *Salmonella* prevalence in grass-fed cattle at slaughter, compared to 10% in grain-fed cattle. Looper et al. (2009) reported just 2% *Salmonella* prevalence in grazing beef cows. It is likely that part of the discrepancy between studies is the time of sample collection, as *Salmonella* prevalence seems to increase following transportation and lairage. Indeed, Schmidt et al. (2015) observed a 5.4% fecal *Salmonella* prevalence rate in cattle at a feedlot, but prevalence increased to 44.6% at time slaughter. Further, differences exist between farms of the same type, within the same region (Edrington et al., 2004, 2008a; Haneklaus et al., 2012; Loneragan et al., 2012). These differences could be due in part to bacterial culture preferences utilized by different laboratories, however they are more likely a function of the wide variability in

Salmonella shedding frequently observed among farms due to factors yet to be elucidated. Clearly, prevalence is a multifaceted phenomenon with substantial variation between year, farm, and group of cattle. Further research investigating the effect of diet, stress, and management is warranted to understand differences in *Salmonella* prevalence within and across cattle types.

Season

It has been commonly reported that warmer months of the year are associated with increased *Salmonella* prevalence in cattle compared to cooler periods (Kunze et al., 2008; Webb et al., 2017; Levent et al., 2019; Nickelson et al., 2019), such as was observed in the present research. The causal mechanism has yet to be fully explained and is generally thought to be a function of elevated temperatures producing more hospitable environmental conditions for *Salmonella* growth and persistence. However, other factors such as seasonal insect vectors and the influence of day length on hormone production are likely involved (Edrington et al., 2006; Pangloli et al., 2008). In the present study, winter presented the time of lowest *Salmonella* prevalence in LN and feces. The seasonal differences in LN and fecal *Salmonella* prevalence is stark, particularly for conventional feedlot and cull dairy cows, suggesting that whatever the effect of warmer weather, it is more impactful on those cattle types. Indeed, Edrington et al. (2004) reported fecal *Salmonella* prevalence of 8% in four dairy farms during in the winter of 2001, but that increased to 41% that summer. Similarly, Edrington et al. (2008b) reported fecal *Salmonella* prevalence of 96% in the summer of 2004 which decreased to 19% that autumn. There may also be a differential effect of season on different breed types, as Nickelson et al. (2019) reported that though they were fattened in the same feedlot, U.S.-raised cattle had a 41% cool season vs. 59% warm season LN *Salmonella* prevalence, while cattle of Mexican origin were unaffected by season (52 and 56%, respectively). It has been shown that heat stress affects tight junction integrity in the intestine (Koch et al., 2019), which may explain a facet of the increased prevalence in LN during warmer months.

Climate Region

Likely by the same mechanism that results in decreased *Salmonella* prevalence during winter, Region 5 (cold) and Region 2 (mixed temperature—humid) had lower *Salmonella* prevalence in the LN while Region 4 (hot—dry) had the greatest in the present study. Comparing LN *Salmonella* carriage between geographical monitoring regions, Gragg et al. (2013b) reported that the Midwest U.S. (cold) was highest for cull cows (4.2%) and Southwest U.S. (hot–dry) was highest for feedlot cattle (19%). In a similar study comparing LN *Salmonella* carriage across three regions [East U.S. (mixed temperatures and humid), Southwest U.S., and West U.S. (mixed temperatures and dry)] in feedlot and cull cattle, Webb et al. (2017) reported that feedlot cattle in the Southwest U.S. had greater prevalence (19%) than in other areas, while cull cattle from the East U.S. had greater prevalence (3.2%) than other areas. Those authors cautioned against inferring too much from regional trends, as weather within a region is unpredictable year-to-year and probably contributes more to *Salmonella* carriage than region does, however it is compelling that such similar results were observed by Gragg et al. (2013b) and Webb et al. (2017), and the present study, in that hot and dry climate regions seem to present conditions for elevated prevalence.

Multi-Drug Resistance

Though overall isolation of MDR *Salmonella* was rare in this study, the greatest incidence of MDR *Salmonella* came from cull beef and dairy cattle. As they are culled animals, it is possible that antimicrobial therapy was administered to the animals prior to culling. Additionally, the increased age of these cattle types compared to feedlot and grass-fed cattle provided more opportunity for them to receive antimicrobial therapy throughout life and therefore more prospect to develop resistance. Supporting the theory of recent or increased antibiotic use in animal classes presenting greater MDR *Salmonella*, Edrington et al. (2008a) reported 45% of fecal samples from sick dairy cows and 58% of fecal samples from hutch calves had tetracycline-resistant *Salmonella*, compared to 10% in healthy heifers and cows. Other studies have reported 5–10% occurrence of MDR *Salmonella* in cull dairy cow fecal samples (Loneragan et al., 2012; Rodriguez-Rivera et al., 2016). Manishimwe et al. (2021) reported only 1% of dairy cattle fecal samples were positive for MDR *Salmonella*, which is more similar to what was found in the present study (1.6%; 10/642). Prevalence of MDR isolates may increase from the time cattle are transported from the feedlot or ranch to the abattoir; Schmidt et al. (2015) reported a 0.5% prevalence rate of 3rd generation cephalosporin-resistant *Salmonella* in fecal samples collected at a feedlot, but that number increased to 1.6% at time of slaughter. More research is needed to understand physiologic changes that occur in times of stress, such as transportation and lairage, that may lead to a rapid increase in prevalence of MDR *Salmonella* in cattle immediately prior to harvest.

CONCLUSION

Similar to other research, results reported herein found that conventional feedlot cattle and cull dairy cows had the highest prevalence of *Salmonella* in both the LN and feces, while surprisingly, all-natural feedlot cattle had the lowest. A primary limitation of this study was that samples were unable to be paired from the same subjects, which could perhaps strengthen future research. In general, results such as these should aid cattle processors that harvest multiple cattle types in a single establishment in risk assessment and abatement. While variation in *Salmonella* is known to exist among similar cattle operations within a region, it is reasonable to assume that concentration of animals on dairies and feedlots, as well as differing feedstuffs and ration formulation, may partly explain why feedlot and dairy animals have the highest *Salmonella* incidence. Future research is underway to better understand the dynamics of *Salmonella* within these cattle types and assess potential pre-harvest intervention strategies.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

LW organized the data, performed statistical analysis, and wrote the manuscript. TE conceptualized and designed the study, gathered data, and revised the manuscript. RA assisted with conceptualization and data interpretation, and revised the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

ACKNOWLEDGMENTS

The authors would like to thank Kate Andrews for her outstanding technical service to complete this research. We also extend our gratitude to the processing plant and its personnel for their hard work in collecting and documenting samples.

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Conflict of Interest: TE was employed by Diamond V Mills, Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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