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Research Paper

Longitudinal Evaluation of *Salmonella* in Environmental Components and Peripheral Lymph Nodes of Fed Cattle From Weaning to Finish in Three Distinct Feeding Locations

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ABSTRACT

Salmonella prevalence in bovine lymph nodes (LNs) varies due to seasonality, geographic location, and feedyard environment. The objectives of this study were to (1) establish prevalence rates of *Salmonella* in environmental components (trough water, pen soil, individual feed ingredients, prepared rations, and fecal samples) and LNs from weaning to finish in three feeding locations, and (2) characterize recovered salmonellae. Calves (n = 120) were raised at the Texas A&M University McGregor Research Center; in lieu of beginning the backgrounding/stocker phase, thirty weanling calves were harvested. Of the remaining ninety calves, thirty were retained at McGregor and sixty were transported to commercial feeding operations (Location A or B; thirty calves each). Locations A and B have historically produced cattle with relatively "low" and "high" rates of *Salmonella*-positive LNs, respectively. Ten calves per location were harvested at the conclusion of (1) the backgrounding/stocker phase, (2) 60 d on feed, and (3) 165 d on feed. On each harvest day, peripheral LNs were excised. Environmental samples were obtained from each location before and after each phase, and every 30 d during the feeding period. In line with previous work, no *Salmonella*-positive LNs were recovered from cattle managed at Location A. *Salmonella*-positive LNs (30%) and environmental components (41%) were most commonly recovered from Location B. Of 7 and 36 total serovars recovered from *Salmonella*-positive LN and environmental samples, respectively, Anatum was identified most frequently. Data from this study provide insight into *Salmonella* prevalence differences among feeding locations and the possible influence of environmental and/or management practices at each. Such information can be used to shape industry best practices to reduce *Salmonella* prevalence in cattle feeding operations, resulting in a decreased prevalence of *Salmonella* in LNs, and thus, minimizing risks to human health.

Numerous studies have shown that harborage of *Salmonella* in peripheral lymph nodes (LNs) is a preharvest phenomenon (Edrington et al., 2013; Fedorka-Cray et al., 1998; Gragg et al., 2013; Webb et al., 2017; Xie et al., 2016). While beef cattle do not always exhibit symptoms of salmonellosis, these animals serve as a carrier for pathogenic *Salmonella*, which has the potential to enter the food supply (Mohler et al., 2009). Peripheral LNs are commonly associated with the fat that accompanies lean trimmings destined for ground beef products (Arthur et al., 2008). The U.S. beef industry fully understands that pathogenic *Salmonella* can cause serious illness in humans. Unfortunately, after several years without a salmonellosis outbreak attributed to beef products, two major outbreaks occurred (Centers for Disease Control and Prevention, 2018a, 2018b), highlighting the need for an increased understanding of the relationship between *Salmonella* and beef.

Published data are limited concerning the carriage of *Salmonella* in bovine lymph nodes and the associated management of live beef cattle. Historically, most studies were designed to capture prevalence rates of *Salmonella* in bovine lymph nodes at harvest (Arthur et al., 2008; Brandt et al., 2013; Brichta-Harhay et al., 2012; Gragg et al., 2012, 2013, 2013; Webb et al., 2017). With regard to feedlot environment, previous work has focused on the presence of anaerobic bacteria (Ouwerkerk & Klieve, 2001) and *Salmonella* (Brandt et al., 2013; Fedorka-Cray et al., 1998) in the feces of feedlot cattle, but not the potential impact of management practices on *Salmonella* prevalence in the feedyard environment and related cattle. More recently, how-

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ever, researchers have begun to investigate different aspects of environmental and/or management factors on the presence of salmonellae in the LNs of feeder cattle. For example, work by our team and others has demonstrated that live cattle production conditions impact fecal shedding and *Salmonella* prevalence in LNs (Green et al., 2010; Haneklaus et al., 2012; Li et al., 2015). Edrington et al. (2013) published data explaining the potential for cuts/scratches and biting insects to be a source of *Salmonella* infection in cattle and also investigated the impact of oral *Salmonella* challenges on uptake by LNs (Edrington et al., 2015), both providing direct links to feeding location conditions. Our research team has investigated the impact of cattle source on recovery of salmonellae from the LNs of cattle postharvest and found no difference in prevalence between sources (Nickelson et al., 2019), further highlighting the importance of understanding management and/or environmental factors surrounding the production of fed cattle.

Edrington et al. (2016) determined that *Salmonella* should be completely cleared by approximately 28 days following a single inoculation event, although it should be noted that the authors identified the need for further research with increased time between inoculation and necropsy to verify these findings. These data represent a first step in a critically important journey to better understand single versus repeated exposures, and the sources of such exposures.

Much remains unknown about how the bovine lymphatic system originally acquires the pathogen, or at what frequency cattle are exposed to and subsequently able to rid their system of the pathogen over time. Preliminary data collected by our research team have shown management practices vary among commercial beef cattle feeding operations (unpublished to date), and that salmonellae prevalence can vary by environmental source (Xie et al., 2016). Further, we have identified clear differences between feeding locations regarding *Salmonella* prevalence in the LNs of cattle postharvest (Belk et al., 2018; Haneklaus et al., 2012) as well as an increase in *Salmonella* prevalence coinciding with the duration of exposure to a given production environment (Belk et al., 2018). While each of these studies have added valuable data to the increasing body of knowledge in this area, we have yet to conduct longitudinal sampling of environmental components (water, pen soil, individual feed ingredients, prepared rations, and fecal drop samples) and LNs from specific groups of cattle over time. Therefore, the primary objective of the present work was to establish prevalence rates of *Salmonella* in environmental components and bovine LNs over the course of feeding from weaning to finish in three distinct feeding locations. A secondary objective of this work was to characterize recovered salmonellae to identify potential trends in the occurrence of certain serovars. Results of this work may be used in guiding the implementation of management practices to prevent or mitigate the presence of *Salmonella* in fed cattle. Such an approach could result in a decreased prevalence of *Salmonella* in lymph nodes, thereby, resulting in decreased risks to human health.

Materials and methods

Study animal selection and management. All animal research activities were approved by the Texas A&M Agricultural Care and Use Committee (Animal Use Protocol 2019-017A). Heifer calves (n = 120) were raised at the Texas A&M University McGregor Research Center (McGregor, TX) and were not comingled with cattle from outside locations at any time prior to relocation. Calves were weaned in grass pens at 6 months of age and weighed after 60 d. Calves were stratified by weight, and within group, were randomly assigned to a feeding location.

In lieu of beginning the backgrounding/stocker phase, thirty weanling calves were transported to Rosenthal Meat Science and Technology Center (RMSTC; College Station, TX) for harvest (October 2019). Of the remaining ninety heifers, sixty were transported to one of two commercial feeding operations (thirty calves each) to complete their feeding program, and thirty were retained at the McGregor Research Center. The selected commercial operations (termed Location A and Location B for the purposes of this work) were in different geographical areas of South Texas and historically produced cattle with greatly differing levels *Salmonella* prevalence in lymph nodes (one “high” and one “low”). In two previous studies (Belk et al., 2018; Horton et al., 2021), weanling calves from McGregor returned *Salmonella*-negative LN samples, thus McGregor Research Center was expected to serve as a second “low-prevalence” feeding environment.

At each feeding location (McGregor, Location A, and Location B), heifers were subjected to industry-typical intake practices and were placed in a single pen. Rations were not standardized across locations, as management and feeding practices typical to each location were maintained. Ten heifers from each feeding location were transported to RMSTC for harvest (1) at the conclusion of the backgrounding/stocker phase (April 2020; this phase included 45 d in a preconditioning pen at all locations followed by 120–130 d on pasture at commercial feeding locations; due to concerns with production of adequate forage, calves at McGregor were not turned out on pasture and remained in their pen), (2) after 60 d on feed (June 2020), and (3) after approximately 165 d on feed upon which time market weight was reached (late September/early October 2020). Two heifers did not complete the study. One heifer was euthanized due to injury at the McGregor Research Center. The second heifer exhibited symptoms of heat stress during transport and was discovered dead upon arrival at the RMSTC (College Station, TX); lymph nodes were recovered although weights were not captured for this animal. The final distribution of heifers across locations and feeding stages can be seen in Table 1.

LN collection and processing. On each harvest day, left and right subiliac and superficial cervical lymph nodes were collected (n = 476 total LNs) from each carcass using a sterilized knife and hook. Within animal, left and right sides of each lymph node type were pooled (n = 238 total samples); therefore, two LNs were placed in each sterile sample bag (VWR, Radnor PA), and transported in insulated shipping containers with refrigerant material to the Texas A&M University Food Microbiology Laboratory (College Station, TX). All LNs were fully submerged in ≥95% ethanol (Decon Laboratories, Inc., King of Prussia, PA) and flame-sterilized to remove potential surface contamination before being aseptically trimmed of fat using flame-sterilized forceps and a scalpel. Denuded LNs were again submerged in ethanol and flame-sterilized before being placed into sterile Whirl-Pak filter bags (Nasco, Sandy Springs, GA) and pulverized using a rubber mallet. Pulverized LN samples were stored in refrigerated conditions (~4°C) for no more than 24 h until *Salmonella* prevalence determination and characterization were performed.

Longitudinal sampling of environmental components. Sampling of environmental components (water, pen soil, individual feed ingredients, prepared rations, and fecal drop samples) was conducted in triplicate for evaluation of *Salmonella* presence and identification of serovars (as appropriate). Samples were obtained from the pen where experimental cattle were housed at each of the three feeding locations. Samples were obtained (1) from the weaning pen used for all calves at McGregor (October 2019), (2) prior to the initial placement of calves in preconditioning pens at the start of the backgrounding stage at each feeding location (October 2019), (3) at the conclusion of the preconditioning phase at each location (approximately 30–45 d; November/December 2019), (4) at the conclusion of the backgrounding/stocker phase at each location (April 2020), (5) before placement of stocker calves in feedlot pens (April 2020), and (6) every 30 d after placement (May through October 2020) until market weight was reached (approximately 165 d on feed). Therefore, a total of n = 696 (n = 666 and 30 for microbiological and soil mineral analyses, respectively) environmental samples were collected between October 2019 and October 2020.

Table 1
Distribution of calves across feeding locations and feeding stages[a]

	No. of heifers			
Feeding stage	McGregor	Location A	Location B	Total
1	30	0	0	30
2	10	10	10	30
3	8	10	10	28
4	10	10	10	30
Total	58	30	30	118

[a] Feeding stages were identified as (1) weaning; (2) backgrounding/stocker; (3) 60 d on feed; (4) approximately 165 d on feed. Two heifers did not complete the study.

Per pen at each feeding location, a minimum of 25 mL of water was collected in each of three sterile 50 mL Falcon tubes (Corning Incorporated). Two tubes per pen at each feeding location were used to skim the trough surface. Then, one tube was submerged below the water surface using gloved hands, opened, allowed to fill, and closed before raising the tube from the trough. Lids were wrapped with Parafilm (Bemis) to prevent leaks and were placed, individually, into sterile sample bags (VWR).

From the surface of each study pen, a minimum of 25 g of soil was obtained in triplicate. Pens were visually divided into thirds, and each sample of a triplicate set represented three locations within a third of a pen. In other words, nine pen surface locations were collectively represented by the triplicate sample at a single location for a given sampling event. Each sample of a triplicate set was placed into a sterile sample bag (VWR) generating three bags per feeding location per sample collection.

In addition to *Salmonella* prevalence determination and characterization, pen soil samples were submitted to the Texas A&M AgriLife Extension Soil, Water and Forage Testing Laboratory (College Station, TX) for routine analysis including micronutrients (pH, NO3-N, Conductivity and Mehlich III by ICP P, K, Ca, Mg, Na, S, DTPA ZN, FE, CU, and Mn). To accommodate sample material needs for soil testing, an additional composite sample of approximately 454 g was obtained concurrently from the same nine sampling sites previously described and placed in a single, separate sample collection bag (VWR) per location per sampling event.

A minimum of 25 g of prepared ration was collected in each of three sterile sample bags (VWR) from three separate locations/depths within the feed bunk of each study pen. Individual feed ingredients also were obtained in triplicate from the feed mill at each feeding location. Each sample was again comprised of a 25 g minimum per sterile sample bag, generating three sample bags per component per feeding location for each sample collection event. Prepared ration composition is proprietary and varied by feeding stage and location. A listing of individual feed ingredients by feeding location is provided in Table 2.

Freshly voided fecal samples also were obtained from each in pen in triplicate. Individual animals were observed for defecation, upon which time a minimum of 25 g per freshly voided fecal pat was collected using a plastic disposable cup and/or spoon. One sterile sample bag (VWR) was used per individual freshly voided fecal sample, generating three bags per pen per location for each sample collection event.

Table 2
Individual feed ingredients[a] sampled across feeding locations

Feeding location		
McGregor	Location A	Location B
Milo Hay	Whole Cotton Seed	Cottonseed hulls
Rolled corn	Cottonseed meal	Brewer's grains
Dry distiller's grains	Hay grazer	Rice bran
Mineral premix	Steam-flaked corn	Steam-flaked corn
Molasses	Mineral premix	Mineral premix
	Molasses	

[a] The contribution of each feed ingredient to prepared ration formulations varied by feeding stage and location. Prepared ration formulations are proprietary.

All samples were double bagged, transported in insulated shipping containers with refrigerant material to the Texas A&M University Food Microbiology Laboratory (College Station, TX), and stored in refrigerated conditions (~ 4°C) for no more than 24 h until microbiological analyses were initiated. Soil samples destined for micronutrient determination were placed in labeled foil pans in a thin layer and dried at 65°C for 16–18 h. Dried samples were allowed to cool to room temperature before being placed in a new, sterile sample bag (VWR), and transported at ambient temperature to the soil testing laboratory (College Station) for analysis.

Positive control preparation. For each harvest day, one extra LN was procured from the head of one animal for in-laboratory inoculation as a positive control. For sampling events in which environmental samples were processed, a pure culture positive control without lymphatic tissue was utilized. Propagation of *Salmonella enterica* serovar Typhimurium Lileengen Type 2 (LT 2) culture was performed 48 h before each collection day by transferring a loop of the stored microorganism from a tryptic soy agar (TSA; Fisher Scientific) slant to a fresh 10 mL tryptic soy broth (TSB; Fisher Scientific) tube and incubating aerobically at 37°C for 18–24 h. The culture then was transferred by pipetting 0.1 mL into a tube containing 10 mL TSB before incubating for 15–18 h at 37°C. To fully evaluate the efficacy of preenrichment, selective enrichment, selective/differential plating, and differential media performance for *Salmonella* detection, pulverized positive control LN samples were inoculated with 1 mL of *S. enterica* serovar Typhimurium type 2 (approximately 3-log CFU/mL) following the second subculturing of the microorganism.

***Salmonella* isolation, confirmation, and serotyping.** Under a sterile biological safety hood, pulverized LNs were placed in modified Tryptone Soya Broth (mTSB; Oxoid Ltd) containing Novobiocin selective supplement (20 mg/L; Oxoid Ltd) for preenrichment at a 1:4 ratio by mass. A 1:10 ratio was used for 10 (hay and cottonseed products) or 25 g (all other sample types) aliquots of environmental sample materials placed into sterile Nalgene (Thermo Fisher Scientific) bottles containing mTSB + Novobiocin for preenrichment purposes. Each preenrichment was hand-massaged (LNs) or shaken for 60 s before incubation. Preenrichments were incubated at 42 ± 1°C for 15–24 h. After incubation, all preenriched samples were analyzed for *Salmonella* presence using a Hygiena BAX® System Q7 (Hygiena) following the BAX® System Q7 Ready Reference for Real-Time PCR Assays for *Salmonella* protocol using Part KIT2006 (Hygiena). Five µL of the preenrichment sample was added to 200 µL lysis reagent that contained a protease enzyme. The samples were incubated at 37°C for 20 min, then at 95°C for 10 min using BAX® System Q7 Automated Thermal Blocks (Hygiena). After incubation, samples were placed in cooling blocks (2–8°C) for 5 min before adding PCR tablets to lysed samples. PCR tubes then were allowed to sit in cooling blocks for 10–30 min before carrying out a two-temperature PCR reaction (hold period 94°C for 2 min; 35 cycles of 94°C for 15 s; 72°C for 3 min; 72°C

for 7 min; hold period at 4°C). Amplified samples were detected by agarose gel electrophoresis at 100 V for 25 min. The BAX system contains a positive control within the machine for detection. Positive, negative, and indeterminate results were indicated by a plus-sign (+), minus-sign (−), or question mark (?) on the results file, respectively.

Positive and indeterminate samples as indicated by the BAX system underwent enrichment in selective broth media: (1) Tetrathionate Broth Base, Hajna (TT Hajna; Becton, Dickinson and Company, Sparks, MD) and (2) modified Rappaport Vassiliadis Broth (mRV; Sigma-Aldrich, Saint Louis, MO). Under a sterile biological safety hood, 0.5 ± 0.05 mL of each preenrichment sample was added to 10 mL of TT Hajna broth and 0.1 ± 0.02 mL of each preenrichment sample was added to 10 mL of mRV broth. The enriched samples were incubated at 42°C for 22–24 h. After incubation, each enrichment sample was streaked onto Double Modified Lysine Iron Agar (DMLIA; HiMedia Laboratories Pvt. Ltd.) with the inclusion of Novobiocin selective supplement (HiMedia) and brilliant green sulfa agar (BGS; Becton, Dickinson and Company) using disposable 10 μL loops (VWR). After streaking, plates were inverted and incubated at 35 ± 2°C for 18–24 h.

From selective/differential agar plates, three individual colonies representing typical *Salmonella* morphologies were chosen per sample, if present. Two differential slants (one each of Triple Sugar Iron and Lysine Iron Agar; Sigma-Aldrich Co.) were inoculated with one-half of each selected colony by stabbing the butts and streaking the slants with a sterile needle, generating six slants per sample. The slants were incubated at 35 ± 2°C for 18–24 h. After incubation, slants were observed for *Salmonella* using descriptions for positive samples from MLG 4.10 (United States Department of Agriculture — Food Safety and Inspection Service., 2019). A sample was considered presumptive positive if a colony yielded positive results on both TSI and LIA slants. The remaining half of each presumptive positive colony from the originating DMLIA or BGS plates was streaked onto a TSA (Fisher Scientific) slant, incubated at 35 ± 2°C for 22–26 h, and stored for no longer than two weeks at 4°C for subsequent confirmation testing.

From each presumptive positive sample, three TSA slants were packaged for refrigerated shipping following Texas A&M University Environmental Health and Safety Department instructions. Slants containing the *Salmonella* isolates were shipped overnight to the USDA – Animal and Plant Health and Inspection Service National Veterinary Services Laboratory (NVSL; ISO 17025 accredited; Ames, IA) for confirmation using matrix-assisted laser desorption ionization—time of flight mass spectrometry (MALDI-TOF). One confirmed-positive isolate per slant was serotyped — yielding up to three serotyped isolates per sample.

Statistical analysis. All data were analyzed using JMP Pro software (version 15.2.1, SAS Institute Inc., Cary, NC). For live cattle and carcass weight data, the Fit Model function of JMP was used to produce one-way analysis of variance by feeding stage with feeding location as a fixed effect. When the main effect was significant, least squares means comparisons were conducted using Student's t test at $P = 0.05$. Data from pen soil analyses (nitrate and trace elements) were analyzed as described above, only modified to include the interaction of feeding stage by feeding location, as appropriate. To examine *Salmonella* prevalence data from LN and environmental component samples, contingency tables were produced for feeding locations and feeding stages and within-table differences were determined using Fisher's exact test for all pairwise comparisons (McGregor vs. Location A, Location A vs. Location B, etc.), and the Bonferroni correction for multiple tests was applied to determine significant differences between pairs.

Results and discussion

Mean live steer weights and carcass weights are presented in Table 3. At the conclusion of feeding stage 2, live and carcass weights for McGregor and Location B heifers were heavier ($P < 0.0001$) than those from Location A. Mean live weights at the conclusion of stage 3 were similar ($P > 0.05$) across feeding locations although resulting mean carcass weights for cattle harvested from Location A were lighter ($P = 0.0298$) than from Location B. No differences ($P > 0.05$) were identified between locations after stage 4 for mean live or carcass weights. These results are to be expected, as similar trends were noted by Belk et al. (Belk et al., 2018) across feeding stages for steers from Locations A and B.

Percentages of *Salmonella*-positive LNs across feeding stages and locations are presented in Table 4. Although 5.0% of samples from weanling heifer carcasses returned *Salmonella*-positive results, all other LN samples analyzed from cattle managed at McGregor were found to be *Salmonella*-negative. This is the first time our team has recovered *Salmonella*-positive LN samples from cattle originating from McGregor as Belk et al. (2018) and Horton et al. (2021) both reported 0% *Salmonella*-positive samples from weanling and feeder-aged calves, respectively. As expected, LNs samples obtained from carcasses of cattle managed at Location A returned low proportions (0/20 LN samples per stage) of *Salmonella*-positive LN samples across all feeding stages. These findings are in agreement with Belk et al. (2018) and Haneklaus et al. (2012) whom both reported 0% *Salmonella*-positive LN samples from the carcasses of cattle managed at Location A. At Location B, 35.0 (7/20), 20.0 (4/20), and 35.0 (7/20)% *Salmonella*-positive samples were confirmed from stages 2, 3, and 4, respectively. Overall, 30% of LN samples from Location B returned a *Salmonella*-positive result. This value is lower than the total percentage of positive LN samples recovered from cattle managed at Location B by Haneklaus et al. (2012) and Nickelson et al. (2019) for finished cattle (42.9% and 52.0%, respectively), or Belk et al. (2018) for cattle across all stages of feeding (64.8%). Additionally, Belk et al. (2018) noted a significant increase in *Salmonella*-positive results from Location B when comparing feeding stage 2 to later stages of feeding, although stages 3 and 4 did not differ ($P > 0.05$) in our current work. Additionally, *Salmonella*-positive results did not differ ($P > 0.05$) across feeding stages for Location B. However, higher proportions of *Salmonella*-positive LN samples were seen for stages 2 and 4 when compared to other feeding

Table 3
Least squares means ± SE for live and carcass weights (kg) by location for each feeding stage[a]

	n[b]	Mean live weight (kg)	Mean carcass weight (kg)
Stage 1			
McGregor	30	196.6 ± 4.6	110.1 ± 3.1
Stage 2			
McGregor	10	378.6 ^{A} ± 8.9	227.4 ^{A} ± 5.6
Location A	10	285.4 ^{B} ± 8.9	168.7 ^{B} ± 5.6
Location B	10	366.2 ^{A} ± 8.9	218.2 ^{A} ± 5.6
P value	-	<0.0001	<0.0001
Stage 3			
McGregor	8	440.7 ± 14.6	267.7 ^{AB} ± 9.3
Location A	10	412.0 ± 13.0	245.4 ^{B} ± 8.4
Location B	10	440.0 ± 13.0	278.5 ^{A} ± 8.4
P value	-	0.2375	0.0298
Stage 4			
McGregor	10	531.1 ± 14.7	336.9 ± 9.5
Location A	10	513.6 ± 14.7	330.7 ± 9.5
Location B	10	534.7 ± 14.7	342.3 ± 9.5
P value	-	0.5609	0.6929

A, B: Values within a column and feeding stage lacking a common letter differ ($P < 0.05$).

[a] Feeding stages were identified as (1) weaning, (2) background/stocker, (3) 60 d on feed, (4) approximately 165 d on feed. Two heifers did not complete the study.

[b] At the conclusion of each feeding stage, heifers from each location were harvested at the Rosenthal Meat Science and Technology Center (College Station, TX).

Table 4
Percentage of Salmonella-positive peripheral lymph node (LNs) samples[a] by location for each feeding stage[b]

Location	Stage 1	Stage 2	Stage 3	Stage 4	Overall
McGregor	5.0 (3/60) X (n = 30 heifers)	00.0 (0/20) A,X (n = 10 heifers)	00.0 (0/18) A,X (n = 9 heifers)	00.0 (0/20) A,X (n = 10 heifers)	2.5 (3/118) A (n = 59 heifers)
Location A	–	00.0 (0/20) A,X (n = 10 heifers)	00.0 (0/20) A,X (n = 10 heifers)	00.0 (0/20) A,X (n = 10 heifers)	00.0 (0/60) A (n = 30 heifers)
Location B	–	35.0 (7/20) B,X (n = 10 heifers)	20.0 (4/20) A,X (n = 10 heifers)	35.0 (7/20) B,X (n = 10 heifers)	30.0 (18/60) B (n = 30 heifers)

A,B: Values within a column lacking a common letter differ ($P < 0.017$) per Fisher's Exact Test and the Bonferroni correction for multiple comparisons.
X: Values within a row sharing a common letter did not differ per Fisher's Exact Test and the Bonferroni correction for multiple comparisons (McGregor, $P > 0.0083$; Location A and Location B, $P > 0.017$).
[a] At the conclusion of each feeding stage, heifers from each location were harvested and left and right superficial cervical and subiliac LNs (n = 476 LNs) were removed. Within animal, left and right LNs of each type were pooled (n = 238 total samples). LNs were not obtained from one heifer.
[b] Feeding stages were identified as (1) weaning, (2) background/stocker, (3) 60 d on feed, (4) approximately 165 d on feed.

locations; this agrees with the assessment of *Salmonella*-positive LNs across feeding stages and locations performed by Belk et al. (2018).

The percentage of *Salmonella*-positive environmental component samples are presented by component and feeding location in Table 5. Overall, the rate of *Salmonella*-positive environmental samples differed by location ($P < 0.017$). McGregor returned the lowest rate (11%), followed by Location A (23.6%), and Location B at 41.0%. At McGregor, *Salmonella*-positive samples were most often collected from pen soil (33.3%) and trough water (42.4%), while *Salmonella* was rarely (3.7% and 0.8% for feces and individual feed ingredients, respectively) or never (0%; prepared ration from the bunk) recovered from samples in the other environmental component categories. *Salmonella*-positive samples were recovered for all sample types at both Locations A and B. The largest ($P < 0.017$) quantity of *Salmonella*-positive pen soil and freshly voided fecal samples were recovered from Location B. *Salmonella* presence in individual feed components collected from each location prior to ration mixing varied ($P < 0.017$) based on location. *Salmonella*-positive individual feed ingredients were least common at McGregor (0.8%), occasional at Location B (12.1%), and recovered most frequently from Location A (28.2%). This trend does not translate directly to prepared rations as sampled from the feed bunk. The highest percentage discrepancy between these two categories is seen for Location B, as prepared ration samples returned *Salmonella*-positive results 46.2% of the time. This differs ($P < 0.017$) from the 0% *Salmonella*-positive sample recovery documented for McGregor, but not ($P > 0.05$) from Location A (24.2%). These findings were somewhat expected based on preliminary data (unpublished to date) for which a larger number of *Salmonella*-positive peripheral and mesenteric LN, pen soil, freshly voided fecal, and trough water samples were recovered from Location B compared to Location A, while the inverse was true for bunk feed samples.

Edrington et al. (2015, 2013) described the need for a "substantial" (10^{10}) oral challenge of *Salmonella* introduced to cattle to ensure recovery of *Salmonella*-positive peripheral LNs. Because 10^{10} is not thought to be a common and naturally occurring *Salmonella* concentration, Edrington et al. (2015) also tested lower oral challenge concentrations over a short time frame although poor recovery of *Salmonella*-positive peripheral LNs was noted. However, a linkage between the gastrointestinal tract and LNs of cattle has been documented by McClelland et al. (2018). It is not fully understood if consistent oral exposure to low *Salmonella* concentrations over an extended timeframe, such as finishing feeder cattle, would result in a higher rate of *Salmonella*-positive LNs from the resulting carcasses of those cattle. It does appear, however, that the cattle managed at Location B face increased exposure to *Salmonella* challenges in general. For example, although not significantly different from Location A, the largest number of *Salmonella*-positive feed samples from the bunk was recovered from Location B. Additionally, a greater number of *Salmonella*-positive samples were recovered from Location B for freshly voided feces, pen soil, and subsequently, LNs.

There are several factors presumed to drive differences observed in *Salmonella*-positive environmental components across commercial beef cattle feeding operations. One established consideration is geographical location (Gragg et al., 2013; Webb et al., 2017). While all three feeding locations evaluated in this study are located within the state of Texas, two of the three feeding locations are over 650 km apart, with natural fluctuations in temperature and rainfall. Belk et al. (2018) reported more than 325 km distance between Locations A and B as well as lower precipitation totals and higher temperatures at Location A compared to B. In addition to weather, populations of wildlife, birds, flies, and other biting/sucking insects can vary regionally and are known to impact *Salmonella* prevalence in LNs of beef cattle (Carlson et al., 2011; Edrington et al., 2013; Olafson et al., 2016, 2014). When surveyed to identify risk factors associated with *Salmonella* in concentrated livestock operations, responses from researchers and feeding operation management revealed large fly populations in pens and around stored manure as the highest risk for beef feedlot environments (Vanselow et al., 2007). While not within the scope of the work presented herein, subsequent experiments designed to identify and evaluate the impact of fly activity and *Salmonella* presence at each feeding location could provide impactful context to the disparity between *Salmonella*-positive sample recovery from individual ingredi-

Table 5
Aggregate percentages of Salmonella-positive environmental component samples[a] for all feeding stages[b] at each feeding location

Location	Freshly Voided Feces	Individual Feed Ingredients	Pen Soil	Prepared Ration from Bunk	Trough Water	Overall
McGregor	3.7 (1/27) B	0.8 (1/126) C	33.3 (11/33) B	0.0 (0/27) B	42.4 (14/33) A	11.0 (27/246) C
Location A	9.5 (2/21) B	28.2 (29/103) A	13.8 (4/29) B	24.2 (8/33) A	26.5 (9/34) B	23.6 (52/220) B
Location B	76.2 (16/21) A	12.1 (12/99) B	85.2 (23/27) A	46.2 (12/26) A	70.4 (19/27) A	41.0 (82/200) A

A,B,C: Values within a column lacking a common letter differ ($P < 0.017$) per Fisher's Exact Test and the Bonferroni correction for multiple comparisons.
[a] Water, pen soil, individual feed ingredients, prepared rations, and fecal drop samples were collected in triplicate from the pen where experimental cattle were housed at each of the three feeding locations. A total of n = 666 environmental samples were collected for the determination of *Salmonella* presences between October 2019 and October 2020.
[b] Feeding stages were identified as (1) weaning, (2) background/stocker, (3) 60 d on feed, and (4) approximately 165 d on feed.

Table 6
Salmonella serovars isolated[a] from bovine peripheral lymph nodes[b] (LN)

Serovar	Stage 1	Stage 2	Stage 3	Stage 4	Total	Percent prevalence
Anatum	0	5	3	3	11	52.38
Lille	0	0	0	4	4	19.05
Havana	2	0	0	0	2	9.52
Agona	0	1	0	0	1	4.76
Cerro	0	0	1	0	1	4.76
Meleagridis	1	0	0	0	1	4.76
Muenchen	0	1	0	0	1	4.76
Total	3	7	4	7	21	100.00

[a] *Salmonella* was isolated following protocols described by Microbiology Laboratory Guidebook 4.10. Three colonies from each presumptive positive sample were selected and packaged for shipment to NVSL (Ames, IA) for confirmation by MALDI-TOF. Confirmed-positive samples were serotyped. Redundant serovars within a sample triplicate were not reported.

[b] Left and right superficial cervical and subiliac LNs (n = 476 LNs) were collected from steers. Within each animal, left and right LNs of each type were pooled (n = 238 samples).

Table 7
Salmonella serovars isolated[a] from environmental component samples[b]

Serovar	Freshly Voided Feces	Individual Feed Ingredients	Pen Soil	Prepared Ration from Bunk	Trough Water	Total	Percent prevalence
Anatum	9	1	9	5	14	38	18.36
6,7:g,m,s:e,n,z15	1	0	9	3	11	24	11.59
Montevideo	1	2	7	1	12	23	11.11
Muenchen	8	1	1	0	7	17	8.21
Mbandaka	0	7	2	2	4	15	7.25
Cerro	2	0	9	0	0	11	5.31
Meleagridis	1	1	7	0	0	9	4.35
Oranienburg	0	5	0	1	2	8	3.86
Senftenberg	0	6	0	2	0	8	3.86
Newport	1	2	2	0	2	7	3.38
Cannstatt	0	3	0	2	0	5	2.42
Jodhpur	1	0	3	1	0	5	2.42
Kentucky	0	0	4	0	1	5	2.42
Liverpool	1	1	1	1	0	4	1.93
Cubana	0	3	0	0	0	3	1.45
Agona	0	1	0	1	0	2	0.97
Gaminara	1	0	1	0	0	2	0.97
Livingstone	0	2	0	0	0	2	0.97
Seftenberg	0	0	0	1	1	2	0.97
Apapa	0	1	0	0	0	1	0.48
Bergen	0	1	0	0	0	1	0.48
Bradenburg	0	1	0	0	0	1	0.48
Fresno	0	1	0	0	0	1	0.48
Godesberg	0	1	0	0	0	1	0.48
Havana	0	1	0	0	0	1	0.48
Idikan	0	1	0	0	0	1	0.48
II 47:b 1,5	0	1	0	0	0	1	0.48
Infantis	0	1	0	0	0	1	0.48
Javiana	0	1	0	0	0	1	0.48
Lexington	0	0	0	1	0	1	0.48
Orion	0	1	0	0	0	1	0.48
Rough O:m,t:-	0	0	0	1	0	1	0.48
Soerenga	0	1	0	0	0	1	0.48
Tennessee	0	0	0	1	0	1	0.48
Typhimurium	0	0	0	0	1	1	0.48
Westhampton	0	1	0	0	0	1	0.48
	26	48	55	23	55	207	100.00

[a] *Salmonella* was isolated following protocols described by Microbiology Laboratory Guidebook 4.10. Three colonies from each presumptive positive sample were selected and packaged for shipment to NVSL (Ames, IA) for confirmation by MALDI-TOF. Confirmed-positive samples were serotyped. Redundant serovars within a sample triplicate were not reported.

[b] Water, pen soil, individual feed ingredients, prepared rations, and fecal drop samples were collected in triplicate from the pen where experimental cattle were housed at each of the three feeding locations. A total of n = 666 environmental samples were collected for the determination of *Salmonella* presences between October 2019 and October 2020.

ents compared to finished rations at these three locations. Factors to consider in the context of fly populations and general hygienic best practices are ingredient and ration storage conditions, time of feed in bunks, and management of feed trucks, manure storage, and pen soil.

In addition to ambient factors, differences in pen design features may have played a role in *Salmonella* recovery from feeding location environments in our study. Although difficult to capture quantitatively, some variance in trough design, pen configuration, available shade, etc. was observed across feeding locations. Most unique was

Table 8
Salmonella serovars isolated[a] from environmental components samples[b] for Location A

Serovar	Freshly Voided Feces	Individual Feed Ingredients	Pen Soil	Prepared Ration from Bunk	Trough Water	Total	Percent prevalence
Oranienburg	0	5	0	1	2	8	13.56
Senftenberg	0	5	0	2	0	7	11.86
Montevideo	0	1	1	0	4	6	10.17
Cannstatt	0	3	0	2	0	5	8.47
Mbandaka	0	4	0	1	0	5	8.47
Newport	1	0	2	0	2	5	8.47
Cubana	0	3	0	0	0	3	5.08
Gaminara	1	0	1	0	0	2	3.39
Livingstone	0	2	0	0	0	2	3.39
Anatum	0	1	0	0	0	1	1.69
Apapa	0	1	0	0	0	1	1.69
Bergen	0	1	0	0	0	1	1.69
Fresno	0	1	0	0	0	1	1.69
Godesberg	0	1	0	0	0	1	1.69
Havana	0	1	0	0	0	1	1.69
II47:b15	0	1	0	0	0	1	1.69
Infantis	0	1	0	0	0	1	1.69
Javiana	0	1	0	0	0	1	1.69
Meleagridis	0	1	0	0	0	1	1.69
Muenchen	0	0	0	0	1	1	1.69
Orion	0	1	0	0	0	1	1.69
Rough O:mt:	0	0	0	1	0	1	1.69
Senftenberg	0	0	0	1	0	1	1.69
Soerenga	0	1	0	0	0	1	1.69
Tennessee	0	0	0	1	0	1	1.69
Total	2	35	4	9	9	59	100.00

[a] *Salmonella* was isolated following protocols described by Microbiology Laboratory Guidebook 4.10. Three colonies from each presumptive positive sample were selected and packaged for shipment to NVSL (Ames, IA) for confirmation by MALDI-TOF. Confirmed-positive samples were serotyped. Redundant serovars within a sample triplicate were not reported.

[b] Water, pen soil, individual feed ingredients, prepared rations, and fecal drop samples were collected in triplicate from the pen where experimental cattle were housed at each of the three feeding locations. A total of $n = 666$ environmental ($n = 220$ from Location A) samples were collected for the determination of *Salmonella* presences between October 2019 and October 2020.

trough construction at feeding location A. Unlike large, immovable troughs observed at McGregor and Location B, troughs at Location A were shallow, resulting in more frequent water turnover. Additionally, troughs at Location A were installed inline with pen fencing and the ability to rotate. Peripheral trough placement discouraged animals from defecating in troughs, and in-line trough rotation facilitated complete water removal and increased ease of cleaning. Additional research designed to evaluate the role of individual management strategies and pen design features could lead to commercial feeding operation best practices for preharvest *Salmonella* mitigation.

Table 6 contains *Salmonella* serovars recovered from peripheral LNs by feeding stage. In total, seven serovars were recovered from LN samples. *Salmonella* Anatum was consistently recovered from feeding stages 2 through 4. Anatum was recovered by Belk et al. (2018) from stage 2 and stage 3 of feeding at Location B, but not 4. *Salmonella* Muenchen is the only other serovar the current work has in common with Belk et al. (2018), although recovered from LNs of cattle harvested at different stages of feeding from Location B for each study. *Salmonella* Anatum is commonly recovered from bovine LNs as documented by our team (Belk et al., 2018; Horton et al., 2021; Nickelson et al., 2019) and others (Gragg et al., 2013; Webb et al., 2017); fortunately, *Salmonella* Anatum is not typically a human illness-causing microorganism (Bosilevac et al., 2009).

Salmonella serovars recovered from environmental components are presented overall (Table 7) and by feeding location (Tables 8 and 9). In total, 36 unique serovars were identified from environmental component samples (Table 7). Six serovars comprised 61.83% (128/207) of total serovars reported, these were Anatum (18.36%), 6,7:g,m,s:e,n,z15 (11.59%), Montevideo (11.11%), Muenchen (8.21%), Mbandaka (7.25%), and Cerro (5.31). Notably, the same six serovars comprise nearly 81% (97/120) of serovars reported for environmental samples from Location B. This equates to over 75% (97/128) of the total for these six serovars isolated from environmental samples across all locations. From McGregor, Meleagridis is the only serovar recovered from both a LN and environmental components (one LN; one feces; seven pen soil). For Location B, three serovars were isolated from both LNs and environmental samples: Anatum (11 LN; 26 environmental), Muenchen (1 LN; 16 environmental), and Cerro (1 LN; 11 environmental). Increased overlap of serovars recovered from LNs and environmental samples would have been unsurprising, as all six of the above-mentioned serovars have been recovered from the LNs of cattle managed at Location B in previous studies (Belk et al., 2018; Horton et al., 2021; Nickelson et al., 2019).

LS means for pH and compound/element (ppm) content of pen soil samples are presented in Tables 9 and 10. Differences ($P < 0.05$) between feeding locations as a main effect were identified for pH and most trace elements (Table 10). Nitrate (ppm) was the only value to differ ($P = 0.0156$) by feeding stage (stage 2 was lowest), although a plausible explanation for this difference is not known to the authors at this time. As seen in Table 10, the only occurrence for which Location B differs ($P = 0.0042$) from McGregor or Location A is for nitrate (ppm). However, in reviewing the laboratory report for this compound at Location B, it is unclear if the value impacting these means was accurate or if laboratory/reporting error occurred, and thus should be interpreted with caution.

A significant interaction between feeding location and feeding stage was present for sulfur, iron, and copper (Table 11). Sulfur content of Location A pen soil was higher ($P = 0.0398$) during stages 3 and 4 compared to stage 2, or any stage at other locations. Mean iron (ppm) values for McGregor and Location A across all stages, as well as stage 2 at Location B, were all similar ($P > 0.05$). However, iron (ppm) in Location B pen soil was higher ($P = 0.0002$) during stages 2 and 3 than any other stage or location combination. A similar phenomenon was inversely identified for mean copper (ppm) values in Location A pen soil. Specifically, copper increased ($P = 0.0017$) in a stepwise fashion across stages at Location A, while all other feeding location

Table 9
Salmonella serovars isolated[a] from bovine peripheral lymph nodes[b] (LN) and environmental components sample[c] for McGregor and Location B

Feeding Location/ Serovar	Lymph Nodes	Freshly Voided Feces	Individual feed ingredients	Pen Soil	Prepared Ration from Bunk	Trough Water	Total	Percent prevalence
McGregor								
Anatum	0	0	0	3	0	8	11	35.48
Meleagridis	1	1	0	7	0	0	9	29.03
6,7:g,m,s:e,n,z15	0	0	0	1	0	6	7	22.58
Havana	2	0	0	0	0	0	2	6.45
Newport	0	0	1	0	0	0	1	3.23
Typhimurium	0	0	0	0	0	1	1	3.23
Total	3	1	1	11	0	15	31	100.00
Location B								
Anatum	11	9	0	6	5	6	37	26.62
Montevideo	0	1	1	6	1	8	17	12.23
Muenchen	1	8	1	1	0	6	17	12.23
6,7:g,m,s:e,n,z15	0	1	0	8	3	5	17	12.23
Cerro	1	2	0	9	0	0	12	8.63
Mbandaka	0	0	3	2	1	4	10	7.19
Jodhpur	0	1	0	3	1	0	5	3.60
Kentucky	0	0	0	4	0	1	5	3.60
Lille	4	0	0	0	0	0	4	2.88
Liverpool	0	1	1	1	1	0	4	2.88
Agona	1	0	1	0	1	0	3	2.16
Senftenberg	0	0	1	0	0	1	2	1.44
Bradenburg	0	0	1	0	0	0	1	0.72
Idikan	0	0	1	0	0	0	1	0.72
Lexington	0	0	0	0	1	0	1	0.72
Newport	0	0	1	0	0	0	1	0.72
Westhampton	0	0	1	0	0	0	1	0.72
Total	18	23	12	40	14	32	138	100.00

[a] *Salmonella* was isolated following protocols described by Microbiology Laboratory Guidebook 4.10. Three colonies from each presumptive positive sample were selected and packaged for shipment to NVSL (Ames, IA) for confirmation by MALDI-TOF. Confirmed-positive samples were serotyped. Redundant serovars within a sample triplicate were not reported.

[b] Left and right superficial cervical and subiliac LNs (n = 476 LNs) were collected from steers. Within each animal, left and right LNs of each type were pooled (n = 238 samples)

[c] Water, pen soil, individual feed ingredients, prepared rations, and fecal drop samples were collected in triplicate from the pen where experimental cattle were housed at each of the three feeding locations. A total of n = 666 environmental samples (n = 246 and 200 for McGregor and Location B, respectively) were collected for the determination of *Salmonella* presences between October 2019 and October 2020.

Table 10
Least squares means ± SE for pen soil analysis results: pH and various compounds/elements (ppm) stratified by feeding location and feeding stage[a]

Main effect	*n*	pH	Nitrate	Phosphorus	Potassium	Magnesium	Sodium	Zinc
Feeding location								
McGregor	9	7.8 ± 0.06 B	61.8 ± 13.1 A	1146.7 ± 174.2 C	2239.3 ± 686.1 B	977.2 ± 111.9 C	303.5 ± 372.2 C	10.9 ± 5.2 B
A	10	8.1 ± 0.06 A	76.5 ± 12.5 A	3317.5 ± 165.9 A	7929.8 ± 653.2 A	2089.8 ± 106.5 A	2841.4 ± 354.3 A	76.5 ± 5.5 A
B	9	7.4 ± 0.06 C	11.3 ± 13.1 B	2065.3 ± 174.2 B	2951.7 ± 686.1 B	1477.2 ± 111.9 B	1243.5 ± 372.2 B	21.5 ± 5.5 B
P-value		<0.0001	0.0042	<0.0001	<0.0001	<0.0001	0.0002	<0.0001
Feeding stage								
2	10	7.7 ± 0.06	67.29 ± 12.5 A	2056.3 ± 165.9	3065.8 ± 653.2	1428.9 ± 106.5	712.5 ± 354.3 B	27.3 ± 5.2
3	9	7.7 ± 0.06	16.2 ± 13.1 B	2051.2 ± 174.2	4974.1 ± 686.1	1520.4 ± 111.9	2120.1 ± 372.2 A	38.2 ± 5.5
4	9	7.8 ± 0.06	66.0 ± 13.1 A	2422.0 ± 174.2	5080.9 ± 686.1	1594.8 ± 111.9	1555.7 ± 372.2 AB	43.4 ± 5.5
P-value		0.4079	0.0156	0.2413	0.0746	0.5677	0.0368	0.1144

A-C: Within a column and main effect, values lacking a common letter differ ($P < 0.05$).

[a] Feeding stages for the study were identified as (1) weaning, (2) background/stocker, (3) 60 d on feed, (4) approximately 165 d on feed. Weaning was only performed at McGregor and is not included in this analysis. Once cattle were placed in feedlot pens, soil samples were collected on d0 and every 30 d thereafter. Stage 3 includes pen soil samples from each location on d0, d30, and d60, and Stage 4 includes samples from d 90 through completion of the feeding period at approximately d 165.

and feeding stage combinations were similar ($P > 0.05$). To date, the authors are unaware of any applicable literature regarding the trace element profile of pen soil in active U.S. feedlots that would allow the appropriate comparison for drawing conclusions from these data.

Previous studies have shown differing levels of *Salmonella* prevalence in bovine lymph nodes due to seasonality (Gragg et al., 2013; Nickelson et al., 2019), geographic location (Gragg et al., 2013; Webb et al., 2017), and feedyard environment (Belk et al., 2018; Haneklaus et al., 2012). Specifically, feeding locations in the southern United States have been a point of concern as this region faces increased *Salmonella* challenges due to southern geography and warm, coastal climate (Belk et al., 2018; Gragg et al., 2013; Webb et al., 2017). With beef-related salmonellosis outbreaks in the U.S., and higher levels of *Salmonella* routinely found and documented in the southern region of the country, working to combat *Salmonella* in lymph nodes of cattle should remain a high priority. This work was designed to help address this need. By overlaying *Salmonella* prevalence in bovine lymph nodes with prevalence in environmental compo-

Table 11
Least squares means ± SE for pen soil values for sulfur, iron, and copper (ppm) stratified by feeding location × feeding stage[a]

Compound/element by feeding location	*n*	Feeding stage 2	3	4
Sulfur				
McGregor	9	144.7 ± 141.7 C	148.0 ± 141.7 C	237.0 ± 141.7 BC
A	10	628.0 ± 122.7 B	1450.7 ± 141.7 A	1508.7 ± 141.7 A
B	9	282.0 ± 141.7 BC	562.7 ± 141.7 BC	603.7 ± 141.7 B
P value	-		0.0398	
Iron				
McGregor	9	16.1 ± 3.0 C	18.3 ± 3.0 C	15.4 ± 3.0 CD
A	10	7.3 ± 2.6 D	16.5 ± 3.0 C	18.4 ± 3.0 C
B	9	12.2 ± 3.0 CD	47.3 ± 3.0 A	31.6 ± 3.0 A
P value	-		0.0002	
Copper				
McGregor	9	2.7 ± 1.1 D	2.2 ± 1.1 D	2.7 ± 1.1 D
A	10	6.8 ± 0.9 C	12.5 ± 1.1 B	15.8 ± 1.1 A
B	9	2.0 ± 1.1 D	1.7 ± 1.1 D	2.7 ± 1.1 D
P value	-		0.0017	

A-C: Within a compound/element, values lacking a common letter differ ($P < 0.05$).

[a] Feeding stages for the study were identified as (1) weaning, (2) background/stocker, (3) 60 d on feed, (4) approximately 165 d on feed. Weaning was only performed at McGregor and is not included in this analysis. Once cattle were placed in feedlot pens, soil samples were collected on d0 and every 30 d thereafter. Stage 3 includes pen soil samples from each location on d0, d30, and d60, and Stage 4 includes samples from d 90 through completion of the feeding period at approximately d 165.

nents associated with general beef production, we can better plan for future research and intervention development and implementation. Data from this study provide insight into *Salmonella* prevalence differences among previously evaluated beef cattle feeding operations and the possible influence of environmental and/or management practices at each. While the goal for both researchers and the industry would be prevention or mitigation of salmonellae uptake by LNs of cattle in commercial feeding environments, a more practical goal of this work is the future development of best practices to reduce *Salmonella* prevalence in cattle feeding operations. This would result in a decreased prevalence of *Salmonella* in lymph nodes, ultimately resulting in decreased risks to human health.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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