TITLE: Comparison of *Salmonella* Enumeration Methods and an Investigation of *Salmonella* Prevalence and Quantification in Market Hog Lymph Nodes and Tonsils in Several Regions and Seasons of the United States

RUNNING TITLE: *Salmonella* market hog lymph nodes and tonsils

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ABSTRACT

Market hog lymph nodes (LNs) can contaminate carcasses with *Salmonella,* as well as ground and comminuted pork products. The objective of this study was to perform a qualitative and quantitative analysis of LNs from several regions and seasons in the United States to establish a *Salmonella* prevalence and concentration baseline. Six types of LNs (axillary, mesenteric, subiliac, tracheobronchial, superficial inguinal, pre-scapular) and tonsils were sampled from market hog carcasses from different regions (east, central, and west) and seasons (winter, spring, and summer/fall). *Salmonella* was detected and enumerated using BAX®-System-SalQuant® methods and the BAX®-System Real-Time *Salmonella* Assay. An enumeration method comparison was also conducted between 3M™EB-Petrifilm™+XLD-replica plates and BAX®-System-SalQuant®. *Salmonella* prevalence (N=4,132) was 36% for tonsils, 35% for mesenteric LN, and less than 10% for the other LN types. Of the 601 carcasses tested, 62% were positive for *Salmonella,* with the highest prevalence occurring during spring in the east (90.9%), and the lowest prevalence occurring during spring in the central region (26.0%). Tonsil prevalence was greatest in the eastern region during spring. Mesenteric LN prevalence was high (>20%) regardless of season or region. *Salmonella* prevalence in tracheobronchial, subiliac, axillary, and superficial inguinal LNs was generally greatest during the spring or fall and in the eastern region. The median SalQuant® *Salmonella* concentration was 2.18 $\log_{10}$ *Salmonella* cells/sample. Median SalQuant® concentration for all other sample types fell below the limit of quantification (1 $\log_{10}$ *Salmonella* cells/sample). Statistical analysis determined that both 3M™ EB-Petrifilm™+XLD-replica plates and BAX®-System-SalQuant® methods could be used for market hog lymph node enumeration. The kappa coefficient between the two methods was 0.384, suggesting fair agreement between the methods, and there was no

significant difference in the mean log for the two methods (p=0.289). This longitudinal study can be used by the pork industry for risk assessments and risk-based decision making.

KEYWORDS: *Salmonella* baseline, pork, lymph nodes, tonsils, United States, *Salmonella* enumeration methods

As of 2021, pork is the second most widely consumed type of meat in the world (Shahbandeh, 2022a), and third-most consumed type of meat in the U.S. (Shahbandeh, 2022b). Researchers have an epidemiological interest in exploring pathogen contamination in pork processing facilities to reduce foodborne illnesses linked to pork products. It was estimated that 110,510,000 metric tons of pork would be produced worldwide in 2022 (Shahbandeh, 2022c) and projected that 122,110 kilotons of pork would be consumed worldwide in 2023 (Shahbandeh, 2022d). However, the United States Department of Agriculture (USDA) estimates that pork products are responsible for more than 500,000 foodborne illnesses each year in the United States (Self et al., 2017). Between 1998 and 2015, there were 288 foodborne outbreaks linked to contaminated pork products (Self et al., 2017). While this data accounts for only a fraction of total foodborne illnesses in the U.S., more research needs to be conducted to reduce the number of foodborne illnesses from pork products.

One of the most common pathogens that causes foodborne illnesses in the world is *Salmonella* (Chaves et al., 2017; WHO, 2022). This Enterobacteriaceae bacterium causes salmonellosis, which can vary in severity depending on host and agent factors (Matthews et al., 2017). Salmonellosis is a medical and economic burden, and the Centers for Disease Control (CDC) estimates that each year *Salmonella* causes 1.35 million illnesses, 26,500 hospitalizations, and more than 400 deaths in the U.S. (2022b).

Between the years 1998 and 2015, there were 72 outbreaks of *Salmonella* attributed to pork products, resulting in 2,215 illness and 276 hospitalizations (Self et al., 2017). According to the CDC, the most recent pork-related *Salmonella* outbreak investigation occurred in 2015 (CDC, 2022a), spanning amongst 5 states with 192 illnesses (CDC, 2015). Research suggests that ground product is at risk of containing contaminated LNs (Arthur et al., 2008; Miller et al.,

2022; Vargas et al., 2023). Lymph nodes are part of the immune system, and the role is to filter and subdue bacteria and viruses found in the body (Arthur et al., 2008; Vargas et al., 2023). During host invasion by *Salmonella*, cells can multiply and spread to LNs, most notably the mesenteric LN due to its location in the viscera (i.e. close proximity to the gut after cells are consumed) (Giannella, 1996). Exposure to *Salmonella* can occur during the harvest and breakdown of the carcass, either by machine or knife once the LN is sliced open. Due to normal methods of pork processing, trim (which consists of fat and muscle tissue) can contain LNs destined for use in comminuted pork (Chaves et al., 2017). For LNs that harbor *Salmonella* and are still encased in adipose tissue, they are protected from chemical and thermal interventions at the abattoir (Gragg et al., 2013; Vargas et al., 2023); thus, rendering normal harvest interventions an inadequate response to this type of contamination. Several studies support the idea of physical removal of the LNs from the carcass to address LN contamination (Bueno López et al., 2022; Jiménez et al., 2023; Miller et al., 2022).

Previous studies investigating *Salmonella* contamination in LNs report a range of prevalence. Miller et al. (2022) reported 34% mesenteric, 18.4% inguinal, 18.3% subiliac, and 16.3% tracheobronchial prevalence (Miller et al., 2022). Chaves et al. 2017 reported a 10% to 44% *Salmonella* prevalence among three pork LN types and tonsils (Chaves et al., 2017). While mesenteric LNs are discarded during processing, other LN types remain in the carcass and untouched by harvest interventions, allowing a possible contamination source for ground product (Vargas et al., 2023).

Rapid pathogen enumeration methods are quickly becoming industry standard; knowing the concentration of pathogens entering processing facilities gives insight into risk of contamination (Mann et al., 2014; Vargas et al., 2023). Polymerase chain reaction (PCR) has

become a more frequent method of pathogen quantification due to time and cost, when comparing to other more traditional methods of quantification, such as most probable number (MPN) or direct plating (Lei et al., 2021; Vargas et al., 2023).

The objectives of this study were to evaluate which LN type presented the highest risk for containing *Salmonella* and at what level of contamination, while also investigating the seasonal and regional variability of *Salmonella* in market hog LNs. Another objective of this study was to compare a direct plating method for enumeration (3M™ EB Petrifilm™ + XLD replica plate) with a rapid PCR method (BAX®-System-SalQuant®). Several authors have published their work related to prevalence among different pork LN types, however there is limited knowledge about regional variation. An even greater knowledge gap exists about seasonal variation in *Salmonella* distribution in market hog lymph nodes. With USDA pork *Salmonella* performance standards forthcoming, this study provides insight into the role of market hog LN *Salmonella* contamination.

MATERIALS AND METHODS

Sample collection. The study was conducted in pork processing facilities located in three regions of the United States, including west, central, and east (Figure 1). A total of five facilities were included in this study. Four facilities were sampled once every season and one facility was sampled twice per season, for three seasons (winter, spring, and summer/fall), for a total of 18 individual sampling events. The winter samplings occurred during February and March 2022; the spring samplings occurred in May and June of 2022; the summer/fall samplings occurred during September and October of 2022, and are hereafter referred to as fall. Seven sample types were collected from every market hog carcass, including mesenteric LN, subiliac LN, superficial inguinal LN, prescapular LN, axillary LN, tracheobronchial LN, and tonsils.

From each plant location during each season, these seven sample types were taken from 30-35 carcasses. During the spring and fall seasons, spleens, cecal swabs, and lairage ropes were also collected when possible. Cecal swabs were collected using cellulose sponges pre-wetted with 25 mL of buffered peptone water (BPW; World BioProducts, Libertyville, IL). Briefly, the cecum was opened, and the sponge was used to swab the interior of the cecum using an in and out motion five times. Sponges were returned to their original sample bag.

All carcasses were USDA Food Safety and Inspection Service (USDA-FSIS) inspected and passed. All samples were collected post-inspection but prior to chilling and placed into individual sterile Whirl-Pak® bags (Nasco, Atlanta, GA). After leaving the harvest floor, samples were placed into insulated containers with cooler packs, and transferred to a 4˚C refrigerator for next day processing at either the K-State Food Safety and Defense Laboratory at Kansas State University or the International Center for Food Industry Excellence (ICFIE) Food Microbiology Laboratory at Texas Tech University.

Lymph node and tonsil processing. Samples were processed and analyzed for *Salmonella* as described in the method development for BAX® system real time PCR (Vargas et al., 2023). Briefly, the LNs and tonsils were trimmed from any surrounding fat and fascia using scissors sanitized before each sample, weighed, and categorized (small: <3 grams; medium: >3 grams and <25 grams). Then, samples were submerged in boiling water (100˚C) for 3-5 seconds for surface sterilization, placed into an individual filtered sterile Whirl-Pak® bag, and pulverized with a rubber mallet. Pre-warmed (42℃) BAX® system MP media (Hygiena, Camarillo, CA) was added aseptically according to weight (small: 20 mL, medium: 80 mL), and subjected to a Smasher (Biomerieux, Marcy-I'Etoile, France) at 620 strokes/min for 1 minute for homogenization. The LN homogenate will be referred to as LNH hereafter.

Cecal swab processing. To each cecal swab bag, 50 mL of pre-warmed (42℃) BAX MP (Hygiena, Camarillo, CA) supplemented with 1mL/1 L of Quant Solution (QS; Hygiena, Camarillo, CA) was added. Sponge sample bags were then homogenized by Smasher for 1 minute and incubated at 42°C for 18-24 hours.

Spleen processing. Spleens were dissected to remove tissue from each end and the center, for a total of 25 grams. Each portion was surface sterilized in boiling water (100˚C) for 3 seconds, placed into an individual filtered sterile Whirl-Pak® bag, pulverized with a rubber mallet, and then homogenized for 1 minute with 100 mL of buffered peptone water (BPW; Hygiena, Camarillo, CA) using a Smasher. Following homogenization, 30 mL of spleen homogenate was transferred to a new Whirl-Pak® bag and combined with 30 mL of pre-warmed (42℃) BAX MP supplemented with 1 mL/1L of QS. Sample bags were hand massaged for 30 s and incubated at 42°C for 18-24 hours.

Rope processing. Ropes were placed into a sterile Whirl-Pak® bag with 300 mL of pre-warmed (42℃) BAX MP supplemented with 1 mL/1 L of QS, hand massaged for 1 minute, and then incubated at 42°C for 18-24 hours.

Salmonella enumeration of lymph nodes and tonsils. Two methods were used to enumerate *Salmonella* from LNs and tonsils: a direct plating method using Enterobacteriaceae (EB) Petrifilm (3M, Saint Paul, MN) + XLD replica plating (following methods described in Vargas et al. (2023)) and BAX®-System-SalQuant® (Hygiena, Camarillo, CA).

For 3M™ EB Petrifilm™+XLD replica plate, a 1 mL aliquot was removed from each LNH to be plated on EB Petrifilm and incubated for 22-26 hours at 37˚C. Characteristic *Salmonella* growth on EB Petrifilm (reddish/purple colonies with acid and gas production) were counted, and then replica plated onto xylose lysine desoxycholate (XLD; Remel, Lenexa, KS) agar plates. The

replica plate was prepared by placing the inoculated film from the EB Petrifilm onto an XLD plate and gently pushing to transfer colonies. The XLD plates were incubated for 16-18 hours at 37˚C andplate counts were recorded by counting characteristic black colonies as *Salmonella*.

For BAX®-System-SalQuant® samples were subjected to the BAX® System Real-time PCR Assay (Hygiena, Camarillo, CA) for *Salmonella* quantification following a 6 hour incubation at 42˚C (Vargas et al., 2023). Sample bags were hand-massaged for 15 seconds before a 5 µl aliquot was removed. The aliquot was processed following manufacturer guidelines for BAX®-System-SalQuant® for *Salmonella* enumeration and concentration was calculated as described by Vargas et al. (2023).

Salmonella detection. Following the enumeration procedures, lymph node and tonsil sample bags were returned to the incubator for another 18 hours at 42˚C. For samples that were not positive during BAX®-System-SalQuant® at 6 hours, twenty-four-hour enrichments were hand massaged for 15 seconds before a 5 µl aliquot was removed and used for BAX® System RT-PCR Assay for *Salmonella* as a detection step.

Spleens, ropes, and cecal fluid samples are reported according to detection only; thus, samples were subjected to a twenty-four-hour enrichment and processed as previously described using the BAX® System RT-PCR Assay for *Salmonella* as a detection step.

Salmonella confirmation and isolation. Samples that were positive for *Salmonella* at 24-hours were subjected to culture isolation. From each sample homogenate, a sterile loop was used to streak for isolation onto XLD agar plates, and incubated for 18-24 hours at 37˚C. Agar plates that contained colonies that were characteristic of *Salmonella* (black, round colonies) were then streaked for isolation on XLD plates, with up to three colonies selected per plate, and incubated for 18-24 hours at 37˚C (Aryal, 2022). One presumptive positive colony from each

plate was inoculated into 10 mL of Tryptic Soy Broth (TSB; BD Diagnostics, Franklin Lakes, NJ), incubated for 18-24 hours at 37˚C, and confirmed for *Salmonella* using BAX® *Salmonella* Real-time PCR Assay with a 5 µl aliquot. Then, 1 ml aliquots with 10% glycerol concentration were saved in a -80˚C freezer for future characterization.

For agar plates with uncharacteristic *Salmonella* growth, or no growth, automatic immunomagnetic separation was performed on the sample homogenate for the respective sample using anti-*Salmonella* beads (Dynabeads; Thermo Sci or Invitrogen, Carlsbad, CA) and a KingFisher™ mL Purification System (Thermo Scientific, Waltham, MA) as described by Miller et al. (2022). The beads (~110 µl) were then transferred into 3 mL of Rappaport-Vassiliadis (Remel, St. Louis, MO) broth, incubated for 18-20 hours at 42˚C, streaked for isolation onto XLD agar plates, and incubated for 18-24 hours at 37˚C. Plates that contained characteristic *Salmonella* colonies (black colonies) were streaked onto XLD and frozen as described above.

Data processing. Detection of *Salmonella* was carried out using the BAX® System using a 24h enrichment. The limit of detection (LOD) was 1 *Salmonella* cell/sample. Each carcass contributed 5~7 sample types and was considered *Salmonella* positive if any of its sampled type was positive. In addition, the number of *Salmonella* positive sample types was tallied for each carcass.

Quantification of *Salmonella* was accomplished using BAX®-System-SalQuant® and XLD at 6h enrichment. The limit of quantification (LOQ) was 10 *Salmonella* cells/sample for BAX®-System-SalQuant® and 0.5 CFU/mL for XLD. Table 1 specifies how the *Salmonella* data enumerated by BAX®-System-SalQuant® were reported.

Statistical analysis for prevalence, enumeration, and method comparison. All tests were conducted at the 0.05 significance level. Comparisons between two levels of a fixed effect

were carried out using two-sided tests. Statistical analyses were performed using the Statistical Analysis Software (SAS 9.4; Cary, NC).

In order to characterize the impact of season and region for each sample type, tthe *Salmonella* prevalence status data were analyzed separately for each sample type. For mesenteric LN, tonsils, and carcass, using the logit linear mixed model. Fixed effects of the model included season, region, and their interaction. The random effect was abattoir visit nested within season and region. Distributions of test statistics were approximated by Chi-square distributions. P-values were obtained via the Wald test. For mesenteric LN, the season-by-region interaction was not significant, and the final model contained main effects only. Fixed effects were evaluated in terms of estimated prevalence rates, their 95% Wald confidence intervals, and odds ratios. Statistical analyses were performed using the SAS GLIMMIX procedure.

The *Salmonella* prevalence data for axillary, inguinal, subiliac, tracheobronchial, and pre-scapular LNs all had overall prevalence rates ≤10%. They were first analyzed by the logit linear model with season and region being the fixed effects. The random effect of abattoir was ignored to assure model convergence and stability. For axillary and tracheobronchial LNs, the deviance/likelihood-ratio test indicated that the season-by-region interaction was significant and the model with main effects only had poor fitness; p-values were then obtained via the Fisher's exact test; the season-by-region effect was evaluated in terms of estimated prevalence rates, their 95% Pearson-Clopper confidence intervals, and odds ratios. For inguinal, subiliac, and pre-scapular LNs, the model with main effects only was adequate and p-values were obtained via the likelihood ratio test; main effects were evaluated in terms of estimated prevalence rates, their 95% Wald confidence interval, and odds ratios. Statistical analyses were performed using the SAS GENMOD and FREQ procedures.

The *Salmonella* enumeration data (quantified by BAX®-System-SalQuant®) were analyzed only for mesenteric LN and tonsil samples that were *Salmonella* positive, as these were the only sample types with enough enumerable samples to be statistically analyzed. Portions of mesenteric LN samples (110 out of 208) and tonsil samples (88 out of 212) fell between the LOD and the LOQ. To account for samples that fell below LOQ, the lognormal accelerated failure time model (i.e., a parametric model for the left-censored data) was employed (Gillespie et al., 2010; Klein & Moeschberger, 2006). Fixed effects of the model included season, region, and their interaction. The random effect of abattoir was ignored. P-values were obtained via the Wald test; interactions were significant and were evaluated in terms of $\log_{10}$ (*Salmonella* cells/sample) least squares means (LSM), their 95% Wald confidence intervals, and difference in LSMs. Statistical analyses were performed using the SAS LIFEREG procedure.

The number of *Salmonella* positive samples per carcass was an ordinal outcome. Data from the 528 carcasses with all 7 sample types collected were analyzed by the cumulative logit linear mixed model. Fixed effects included season, region, and their interaction. The random effect was abattoir visit nested within season and region. Distributions of test statistics were approximated by Chi-square distributions. P-values were obtained via the Wald test. Fixed effects were evaluated in terms of estimated rate for number of positive samples and the cumulative odds ratios for having fewer positive samples. Statistical analyses were performed using the SAS GLIMMIX procedure.

The receiver operating characteristic (ROC) curve plots sensitivity vs. one minus specificity of a model and was used to determine if *Salmonella* levels in various sample types could serve as the sentinel for *Salmonella* prevalence in the mesenteric LN and tonsils. Specifically, the *Salmonella* prevalence status in the mesenteric LN of a carcass was modelled

using the logistic regression with explanatory variables being *Salmonella* levels (either ND, [LOD, LOQ), or ≥LOQ) of the other six sample types from the same carcass; the *Salmonella* prevalence status in tonsils of a carcass was modelled by the *Salmonella* levels of the other six sample types from the same carcass. Hosmer and Lemeshow provided the guideline for the interpreting area under the curve (AUC) of the ROC function as: AUC<0.6, failed prediction; 0.6≤AUC<0.7, poor prediction; 0.7≤AUC<0.8, acceptable prediction; 0.8≤AUC<0.9, excellent prediction; AUC≥0.9, outstanding prediction (Hosmer et al., 2013). Data from the 528 carcasses with all 7 sample types collected was analyzed using the SAS LOGISTIC procedure.

The BAX®-System-SalQuant® and XLD methods (6h enrichment) were compared based on *Salmonella* positive samples (detected by BAX®-System-SalQuant® with a 24h enrichment; tonsils were excluded; n=378). The Cohen's kappa statistic was used to measure the degree of agreement, in terms of the ability to quantify *Salmonella*, between the two methods. Landis and Koch assigned the following benchmarks for agreement strength: poor (<0.00), slight (0-0.20), fair (0.21-0.40), moderate (0.41-0.60), substantial (0.61-0.80) and almost perfect (0.81-1.00) (Landis & Koch, 1977). For samples quantifiable by both methods, the mean $\log_{10}$ (CFU or *Salmonella* cells/sample) difference was evaluated using the one-sample *t*-test for non-zero difference and one-sample confidence interval. Statistical analyses were performed using the SAS FREQ and MEANS procedures.

Prevalence data, including the standard error of the mean, for spleens, cecal fluid, and ropes was calculated using Microsoft Excel (Redmond, WA).

RESULTS AND DISCUSSION

Prevalence and enumeration of lymph nodes and tonsils. A total of 4,132 samples (lymph node and tonsils) were collected in this study; 590 samples were positive for *Salmonella*

when evaluated using the BAX *Salmonella* Real Time PCR Assay detection method. The mesenteric LNs and tonsils had the highest prevalence, at 35% (n=600) and 36% (n=591), respectively. The market hog carcasses had a 62% (n=601) prevalence for *Salmonella*. The overall *Salmonella* prevalence was 14% (n=4,13*2*), with the highest overall prevalence occurring during the spring season (17% [n=1,399]), in comparison to 14% (n=1,359) and 12% (n=1,374) during winter and fall, respectively. The overall *Salmonella* prevalence (14%) in this study was lower than the 21.8% prevalence (samples included mesenteric, inguinal, subiliac, and tracheobronchial LNs) reported by Miller et al. (2022) and the 27.7% (sample types included ileum, ileocolic and mandibular LNs, and tonsils) reported by Vieira-Pinto et al. (2005). In the present study, tonsils had the greatest overall prevalence at 36%, whereas mesenteric LN represented the largest prevalence in studies by Vieira-Pinto et al. (2005) and Miller et al. (2022) at 35% and 34%, respectively. In comparison, Chaves et al. (2017) reported that mesenteric LNs had a 44% and 20% prevalence in two regions in Mexico, whereas the tonsils had an 18% and 40% prevalence in the two regions (Chaves et al., 2017). Variability between studies may be due to a variety of things, including detection methods, sampling region or season, sample numbers, among others.

A season-by-region interaction was detected for *Salmonella* prevalence of axillary LN (P=0.038), tracheobronchial LN (P=0.007), and tonsils (P=0.006) as shown in Figure 2. Figure 2A displays the data with region on the y-axis, while Figure 2B displays the data with season on the y-axis. Among these three sample types, tonsils had the highest prevalence, with the greatest prevalence observed in the eastern region during the spring season (74.2% [n=132]). While tonsils consistently had the highest prevalence during each season when compared to axillary

and tracheobronchial LNs, it is important to point out that LN types were not statistically compared to one another, and this is a general observation.

As shown in Figure 2A, tonsils and the axillary and tracheobronchial LNs varied by season within the eastern region, with prevalence significantly higher ($P \leq 0.05$) in spring for tonsils (74.2%) and tracheobronchial LNs (34.8%). Axillary LN prevalence in the eastern region was highest in spring (15.2%), which was significantly higher ($P=0.009$) than prevalence in fall (1.6%). Tonsils were the only sample type that varied by season in the western region, with winter (57.6%) and spring (44.6%) prevalence both significantly higher ($P \leq 0.05$) than fall (15.8%). These same general trends can be observed in Figure 2B, which illustrates regional variation within season for tonsils, as well as the axillary and tracheobronchial LNs. However, Figure 2B also highlights that axillary prevalence is greatest in the east during winter (10.3%), which is greater than ($P=0.013$) the 0.0% (zero positive samples) prevalence in the western region, but statistically the same ($P=0.063$) as the 1.6% prevalence reported in the central region.

A season-by-region interaction was also observed ($P=0.001$) for *Salmonella* prevalence at the carcass-level. Percent prevalence data for carcasses are displayed in Table 2. In the central region, carcass prevalence varied by season ($P=0.007$) with prevalence significantly higher ($P \leq 0.05$) in winter (55.9%) and fall (51.5%) in comparison to spring (26.0%). In the eastern region, carcass prevalence was significantly higher in spring (90.9%) than winter (76.5%; $P=0.048$) or fall (64.2%; $P=0.002$). In the spring season, *Salmonella* prevalence in market hog carcasses varied by region ($P<0.001$) as follows: 90.9% in the east, 65.2% in the west, and 26.0% in central, and all regions were significantly different than one another ($P \leq 0.05$). The main effect of season was not significant for mesenteric LNs ($P=0.877$) but was significant for superficial inguinal ($P=0.008$), subiliac ($P=0.025$), and pre-scapular ($P=0.022$) LNs (Figure 3A).

In general, mesenteric LNs had the highest prevalence (Figure 3A) at 31.7%, 32.6%, and 36.2% during winter, spring, and fall, respectively. Pre-scapular LN prevalence was lowest in winter (0.8%) when compared to spring (3.2%; P=0.011) and fall (3.0%; P=0.018). The spring subiliac LN prevalence (4.4%) was significantly higher (P=0.010) than winter (1.1%). A similar trend was observed for superficial inguinal LN prevalence, which was significantly greater (P=0.002) in spring (8.9%) in comparison to winter (2.3%). Little is known about the seasonal variability of *Salmonella* in market hog lymph nodes. A seasonal effect has been observed for *Salmonella* prevalence in beef LN studies; prevalence was significantly higher (P=0.0304) in the summer/fall months when compared to the winter/spring months (Gragg et al., 2013). This trend is consistent with the occurrence of human salmonellosis, as the CDC reports cases are more common during the summer months than in the winter (CDC, 2019).

The main effect of region was not significant for mesenteric (P=0.061) or superficial inguinal (P=0.052) LNs but was significant for subiliac (P<0.001) and pre-scapular (P<0.001) LNs (Figure 3B). Prevalence in the eastern region was significantly greater ($P\leq$0.05) for both subiliac (8.5%) and pre-scapular (12.4%) LNs when compared to prevalence in the central and western regions for both LN types. Although LN types were not statistically compared, it should be noted that mesenteric lymph node prevalence was also the highest in each region at 45.6%, 32.5%, and 23.9% in the eastern, western, and central regions, respectively. A study conducted by Bessire et al. (2018) reported a significant difference in *Salmonella* prevalence among sow inguinal LNs between the northern and southern regions (Bessire et al., 2018); though, it is difficult to compare location between these two studies because regions are defined differently. Sows in the northern region had a higher occurrence of *Salmonella*. It is also important to consider that *Salmonella* prevalence likely varies between market hogs (present study) and sows

(Bessire et al., 2018). In a similar beef LN study, it was reported that states located in the southern part of the U.S. had a significantly higher (P=0.0198) *Salmonella* prevalence in subiliac lymph nodes from feedlot cattle than the northern region (Gragg et al., 2013).

The goal of sampling six types of LNs and the tonsils was to aid in market hog carcass mapping of *Salmonella* contamination. Mesenteric and tracheobronchial LNs were sampled to represent contamination within the viscera. Peripheral LNs (such as subiliac and superficial inguinal) were sampled to represent the LN types at risk of being incorporated into final meat products (Miller et al., 2022). Tonsils were used as a marker for contamination by the oral route, including while in lairage and possibly from shedding during transportation (Chaves et al., 2017; Hurd et al., 2001). *Salmonella* was found in each sample type in this study; however, *Salmonella* was not observed in all seven sample types from a single carcass, as shown in Table 3. Table 3 describes the number of carcasses harboring between 0 and 7 positive samples. *Salmonella* was not detected in 38% of carcasses; it was detected in at least one LN in 36% of carcasses, and at least 2 LNs in 17% of carcasses. For the sake of simplicity, tonsils were included in the LN count in Table 3. Figure 4 displays the number of positive sample types by carcass in each season and region, providing another way to view the data provided in Table 3. In general, spring in the eastern region had more positive sample types from a carcass (i.e., the most frequently observable number of 6 positive sample types from one carcass). Miller et al. (2022) analyzed the prevalence data in a similar way to Table 3 (sample types included mesenteric, inguinal, subiliac, and tracheobronchial LNs, all of which were sampled in this current study). *Salmonella* was not detected in 38% of the carcasses, but none of the carcasses contained *Salmonella* in all four LN sample types. Overall, 40% of the carcasses had *Salmonella* in one LN type (Miller et al., 2022). The results described by Miller et al. (2022) are very similar to the results displayed in

Table 3, where *Salmonella* was not detected in any LN for 38% of the carcasses in both studies. Moreover, in both Miller et al. (2022) and the present study, *Salmonella* was not observed in all four or seven sample types from a single carcass.

A complete set of all seven samples (six LNs and tonsils) was collected from a total of 528 carcasses throughout this study. The *Salmonella* prevalence for these 528 carcasses was analyzed according to number of samples positive per carcass, and a season-by-region interaction (P=0.034) was observed. In the eastern region, carcasses sampled in the winter and fall were more likely ($P \leq 0.05$) to have a fewer number of samples positive for *Salmonella* than carcasses sampled in the spring. During the spring season, all regions were significantly different ($P \leq 0.05$), with more positive samples collected from carcasses sampled in the east, followed by the west, and then central.

Excluding tonsils, 378 LNs were positive for *Salmonella*, of which 150 were quantifiable using BAX®-System-SalQuant® while the other 228 samples fell below the LOQ (meaning the samples were positive for prevalence, but not able to be quantified). Enumeration median values varied depending on sample type; not all sample types were able to be quantified, with all median *Salmonella* enumeration values falling below the LOQ, except for tonsils. The *Salmonella* enumeration data were analyzed only for mesenteric LN and tonsil samples that were *Salmonella* positive because these were the only sample types with enough positive samples at concentrations above the LOQ to statistically analyze. Several mesenteric LN samples (110 out of 208) and tonsil samples (88 out of 212) harbored *Salmonella* at concentrations between the LOD and the LOQ.

A season-by-region interaction for *Salmonella* concentration in mesenteric LNs (P=0.014) and tonsils (P=0.031) was significant (Figure 5). In general, concentration of

392 *Salmonella* appears to be the greatest during the winter season for mesenteric LNs, though
393 concentration did not vary by region during this season (P>0.05). During the spring season, the
394 *Salmonella* concentration in mesenteric LNs from the western region (2.2 $\log_{10}$ *Salmonella*
395 cells/sample) was significantly greater (P=0.034) than in the central region (1.1 $\log_{10}$ *Salmonella*
396 cells/sample). Mesenteric LN *Salmonella* concentration in the fall was significantly greater
397 (P=0.013) in the eastern region (1.8 $\log_{10}$ *Salmonella* cells/sample) than in the western region
398 (0.7 $\log_{10}$ *Salmonella* cells/sample). Median *Salmonella* concentration for mesenteric LNs fell
399 below the LOQ for BAX®-System-SalQuant®. *Salmonella* concentration in tonsils during the
400 winter in the western region was 3.0 $\log_{10}$ *Salmonella* cells/sample, which was significantly
401 greater (P=0.001) than the 1.5 $\log_{10}$ *Salmonella* cells/sample observed in the central region
402 during the same season. *Salmonella* concentration in tonsils also varied during the fall season,
403 with the eastern region (2.8 $\log_{10}$ *Salmonella* cells/sample) and western region (2.7 $\log_{10}$
404 *Salmonella* cells/sample) significantly greater (P>0.05) than the central region (1.1 $\log_{10}$
405 *Salmonella* cells/sample). The median *Salmonella* concentration for tonsils was 2.18 $\log_{10}$
406 *Salmonella* cells/sample.

407 Evaluation of *Salmonella* concentration in market hog lymph nodes is limited, especially
408 in comparison to beef lymph nodes. Recently, *Salmonella* enumeration was explored in four LN
409 types (mandibular, superficial inguinal, superficial popliteal, and medial iliac) where most
410 enumeration values from positive samples were below 0.66 $\log_{10}$ MPN/g. However, the highest
411 concentrations of *Salmonella* were 3.9 and 3.7 $\log_{10}$ MPN/g (Larsen et al., 2023). Mesenteric
412 LNs from beef cattle have been found to contain up to 5000 CFU/g of *Salmonella* (Samuel et al.,
413 1980; Vargas et al., 2023). Moreover, another beef lymph node study reported a mean
414 *Salmonella* concentration of 1.75 $\log_{10}$ CFU/g, with a range up to 3.8 $\log_{10}$ CFU/g (Gragg et al.,

2013). Furthermore, peripheral beef LN *Salmonella* concentrations have been reported to range from 1.6 to 4.9 $\log_{10}$ CFU/LN (Webb et al., 2017). These studies use different enumeration methods, but all highlight the importance of exploring *Salmonella* concentration in LNs because they can contaminate comminuted product.

It is necessary to discuss the shortcomings of using the LN BAX®-System-SalQuant® curve to describe the tonsil results; as mentioned in the method comparison discussion above, tonsils harbored substantial background microflora that interfered with the 3M™ EB Petrifilm™+XLD replica plate enumeration method. Because of this, a separate BAX®-System-SalQuant® curve should be developed as a quantification method specifically for tonsils. Future research conducted on market hog tonsils should take this into account because the LN BAX®-System-SalQuant® quantification curve used in this study may not be an accurate method for tonsil quantification, and the concentration data presented herein for tonsils should be interpreted accordingly.

It is important to discuss that only mesenteric LNs and tonsils had enough positive samples with *Salmonella* concentrations above the LOQ to be enumerated. Mesenteric LNs and tonsils aren't typically destined for comminuted pork and are, instead, disposed of at the plant, so these data are useful in understanding contamination within the gastrointestinal tract (Miller et al., 2022). The risk of *Salmonella* contamination of ground pork from these two sample types is limited in comparison to the peripheral LNs that are in adipose tissue, and therefore risk to public health is limited as well.

Prevalence of spleens, ropes, and cecal fluid. Overall *Salmonella* prevalence for cecal fluid, ropes, and spleens was 38.3% (N=269), 95.5% (N=22), and 9.2% (N=336), respectively. Table 4 summarizes the prevalence of each sample type according to sampling season and

region. These data were not statistically analyzed to determine the effect of season or region because of the inconsistency in collection of these sample types, which included several instances of missing data within a particular region or season. In general, ropes hung in lairage pens regularly harbored *Salmonella*, which may be an indication of oral contamination that may be the result of exposure in lairage; however, it is important to note that lairage ropes often come into contact with the body as pigs walk through the pen. *Salmonella* prevalence in cecal fluid was notably high at 71.2% in pigs from western region during the fall months. In general, *Salmonella* contamination within spleens was <10%, apart from a 26.5% prevalence recorded during the spring in the eastern region.

Assessing risk. Table 6 summarizes the overall prevalence and concentration for tonsils and each lymph node. Based upon these data, tonsils and mesenteric lymph nodes present the greatest risk; however, these sample types are not destined for ground product and are often discarded, and their risk within an abattoir is more associated with cross-contamination with an abattoir. Tracheobronchial lymph nodes would be considered low risk based upon their prevalence and concentration, but these lymph nodes are also discarded with the viscera.

While axillary, pre-scapular, subiliac, and superficial inguinal lymph nodes may be incorporated into ground product, the overall prevalence associated with these sample types was less than 10% and their median concentration was less than the 1 log *Salmonella* cells/sample limit of quantification. This would suggest that axillary, pre-scapular, subiliac, and superficial inguinal lymph nodes are of lower risk for ground product contamination. While risk may seem low overall, it is important to note that the maximum concentration recorded for lymph nodes that are more associated with ground product was 3.0, 5.1, 5.3, and 4.6 log *Salmonella* cells/sample for axillary, superficial inguinal, subliac, and pre-scapular, respectively.

Using a threshold of 10% *Salmonella* prevalence to assess risk, axillary lymph nodes collected from carcasses in the eastern region during winter (10.3%), but particularly during spring (15.2%), would be considered of greater risk than other region and season combinations. Similarly, pre-scapular lymph nodes collected from carcasses in the eastern region harbored *Salmonella* at a prevalence of 12.5%. *Salmonella* prevalence in subiliac and superficial inguinal lymph nodes did not exceed the 10% prevalence threshold for risk.

Method comparison. One of the objectives of this study was to compare 3M™ EB Petrifilm™+XLD replica plate method to BAX®-System-SalQuant®. Table 6 shows the number of samples that were *Salmonella* positive as determined by each methodology. Tonsils were not included in this comparison. More specifically, tonsils were removed from the method comparison because the 3M™ EB Petrifilm™+XLD replica plate data were collected during only one season. The background growth from other EB colonies was interfering with proper enumeration for tonsils; thus, the 3M™ EB Petrifilm™+XLD replica plate method was abandoned for tonsil enumeration. Similarly, the pork LN BAX®-System-SalQuant® curve is not the proper method to evaluate *Salmonella* enumeration for tonsils. The heavy background flora in tonsils likely interfered with proper enumeration of these samples; thus, tonsil enumeration data should be interpreted accordingly. It is recommended that a curve specific for tonsils is developed and used for pork tonsil enumeration.

The contingency table (Table 6) displays the number of samples that were categorized from each methodology. The kappa coefficient between 3M™ EB Petrifilm™+XLD replica plate method and BAX®-System-SalQuant® is 0.384, which indicates a fair agreement between the two methodologies. Of the 378 samples that were positive and quantifiable for *Salmonella*, 3M™ EB Petrifilm™+XLD replica plate method and BAX®-System-SalQuant® were both able

484 to both quantify 83 samples out of 378 *Salmonella* positive samples. The 3M™ EB
485 Petrifilm™+XLD replica plate method and BAX®-System-SalQuant® methods were both able to
486 determine that 183 out of 378 samples lie below the LOQ. The 3M™ EB Petrifilm™+XLD
487 replica plate method quantified 45 samples that were not quantifiable by BAX®-System-
488 SalQuant® and, conversely, the BAX®-System-SalQuant® quantified 67 samples that were not
489 quantifiable by the 3M™ EB Petrifilm™+XLD replica plate method. The blue dots on Figure 6A
490 represent *Salmonella* positive samples that were quantifiable by BAX®-System-SalQuant® but
491 were below the LOQ for the 3M™ EB Petrifilm™+XLD replicate plate method. The green dots
492 represent positive samples that were quantifiable by both methodologies. The limit of
493 quantification for the 3M™ EB Petrifilm™+XLD replicate plate method is determined by
494 sample weight and volume of media added for enrichment. BAX®-System-SalQuant® has a
495 lower LOQ, which is 1 log *Salmonella* cells/sample, or 10 *Salmonella* cells/sample. The blue
496 dots located on the left side of the graph in Figure 6A correspond with the limit of quantification
497 that was described by Vargas et al. (2023).
498 For the 3M™ EB Petrifilm™+XLD replicate plate method, the lowest value that can be
499 estimated is 1 CFU/2 mL (0.5 CFU/mL) because of duplicate plating. For small LNs (<3 grams),
500 20 ml of BAX MP media was used to enrich, with a sample weight on average being 2 grams,
501 which would result in ~10 CFU/sample lower limit (0.5 CFU/g * 22 total g in sample bag = 11)
502 for estimation. This same process can be used to determine the lower limit for medium sized LNs
503 (>3 grams); for 80 ml of media and an average sample weight of 10 to 20 grams, the limit would
504 be ~50 CFU/ml (0.5 CFU/g * 100 total g in sample bag = 50). The $\log_{10}$ conversion of these
505 values is 1 log CFU/sample and 1.7 log CFU/sample.

Addressing the issues of background growth and dilutions were the largest disadvantages in this study when comparing the 3M™ EB Petrifilm™+XLD replicate plate method to BAX®-System-SalQuant®. In instances where too many colonies to count were observed on 3M™ EB Petrifilm™, a dilution would be performed to obtain a countable range for *Salmonella*; however, *Salmonella* colonies were often not enumerable on XLD plates once a countable range was determined on the 3M™ EB Petrifilm™ plate. The EB Petrifilm™ plate enumerates all members of the Enterobacteriaceae family; thus, background growth on the 3M™ EB Petrifilm™ prevented proper quantification; however, these samples were still positive for *Salmonella* according to BAX®-System-SalQuant®. Another variable affecting the stacks of blue dots in Figure 6A is the dilution factor used to obtain a countable range on 3M™ EB Petrifilm™+XLD replicate plate. The stack of blue dots located on the left side of Figure 6A would represent samples below the LOQ with dilution factors of 0 or 1. The stack of blue dots on the right side of the figure represent samples below the LOQ with dilutions of 2 or 3. The quantification values from samples that were diluted on the 3M™ EB Petrifilm™ shown in Figure 6A do not represent actual numbers, but rather these values have been extrapolated to represent quantification values when background flora limits the ability for proper enumeration. The methods used to calculate the level of *Salmonella* from a 3M™ EB Petrifilm™ plate that has been diluted estimates a number, meaning the corresponding blue dots are estimates that lie below the limit of quantification for the 3M™ EB Petrifilm™+XLD replicate plate method.

In Figure 6B, the t-test conducted on the 83 samples that were quantifiable by both methods resulted in a p-value of 0.289, suggesting that there was no significant difference in the mean log for either method. The distribution curve in Figure 6B also shows that most samples were enumerated between the ranges of 1 and 3 logs. Out of the roughly 4,000 samples collected,

529 only 4 LN samples were quantified at a level above 6.5 logs of *Salmonella* using 3M™ EB
530 Petrifilm™+XLD replica plate method. According to Teunis et al. (2010), 4 logs of *Salmonella*
531 is concentrated enough to cause a 50% chance of human illness (Teunis et al., 2010); however,
532 salmonellosis is also influenced by host immunity, as well as serotype, virulence factors, and the
533 infectious dose associated with a particular *Salmonella* strain (Lozano-Villegas et al., 2023). It is
534 important to note that LNs are not directly consumed, rather they are mixed into batch of ground
535 pork (i.e., thousands of pounds of product), resulting in a dilution effect. It must be
536 acknowledged that there won't be an even distribution of *Salmonella* contamination within the
537 batches of ground pork, so the true dilution effect is unknown. The risk with ground pork
538 contamination lies with the number of LNs that go into the grind, as well as the number of those
539 LNs that are contaminated, recognizing that the prevalence data presented herein demonstrate
540 that not all LNs harbor *Salmonella*. There are several factors of *Salmonella* that will determine
541 the effect on human health such as serotype, virulence factors, and dosage of bacteria. The
542 amount of *Salmonella* in a sample and/or batch of ground pork will determine the risk of human
543 infection. Lower concentrations of *Salmonella* are not as large of a risk in comparison, but can
544 still cause foodborne illness (Teunis et al., 2010).
545 The 3M™ EB Petrifilm™+XLD replica plate method and BAX®-System-SalQuant®
546 method of quantification can be used to enumerate *Salmonella,* as the t-test for the samples that
547 were quantified with both methods showed no significant difference in methodologies for
548 quantification. Determining which method to use will vary depending on laboratory and protocol
549 because the 3M™ EB Petrifilm™+XLD replicate plate and BAX®-System-SalQuant® methods
550 each have advantages and disadvantages. For the 3M™ EB Petrifilm™+XLD replica plate
551 method, approximately 2 days are required to obtain data. During this process, there is room for

552 human error, such as when removing the aliquot from the sample bag, plating on the Petrifilm™
553 media, during replica plating onto XLD plates, and counting colonies. Another disadvantage to
554 the 3M™ EB Petrifilm™+XLD replica plate method is the counting of black colonies on the
555 XLD plates; while XLD is a selective media most frequently used for EB and *Salmonella*, not all
556 *Salmonella* will grow as black colonies, and not all black colonies are *Salmonella*. The 3M™ EB
557 Petrifilm™+XLD replica plate method performs well at enumerating higher logs of *Salmonella*,
558 such as values greater than 3 logs, as described by Vargas et al. (2023). In comparison, the
559 BAX®-System-SalQuant® method takes 6 hours for incubation, with 75 minutes post-incubation
560 to receive results. The BAX®-System-SalQuant® method determines absence (negative) or
561 presence (positive) of a gene in *Salmonella*, which is more reliable than counting black colonies
562 on XLD plates. A disadvantage of using BAX®-System-SalQuant® may be the aliquot size;
563 BAX®-System-SalQuant® uses 5 microliters for quantification, in comparison to the 1 mL used
564 for the 3M™ EB Petrifilm™+XLD replica plate method. Furthermore, there are differences in
565 limits of quantification between the two methodologies. The BAX®-System-SalQuant® has a
566 predetermined lower limit of 10 cells, while 3M™ EB Petrifilm™+XLD replica plate method
567 has a higher limit of 10 to 50 CFU (depending on sample size). Vargas et al. (2023) displays the
568 linear correlation of the 3M™ EB Petrifilm™+XLD replica plate method and BAX®-System-
569 SalQuant® *Salmonella* quantification (Vargas et al., 2023). On average, 3M™ EB Petrifilm™ +
570 XLD replica plate method estimates 3 to 5 logs more accurately, while BAX®-System-SalQuant®
571 estimates 1 to 3 logs of *Salmonella* more accurately, as described by Vargas et al. (2023).
572 There are samples that were similarly quantifiable by both methodologies when
573 considering the green dots in Figure 6A. For example, there are a cluster of green dots
574 surrounding 3 log enumeration for the 3M™ EB Petrifilm™+XLD replica plate method and

BAX®-System-SalQuant®. An individual green dot from Figure 6A that can be specifically discussed is situated at 5.4 logs on the 3M™ EB Petrifilm™+XLD replica plate method axis, and 4.5 log on BAX®-System-SalQuant® axis. While these values are within a 1 log difference, we should expect the 3M™ EB Petrifilm™+XLD replica plate enumeration to be more accurate (Vargas et al., 2023). Conversely, BAX®-System-SalQuant® enumerated one sample in Figure 6A at 2.2 logs, while the 3M™ EB Petrifilm™+XLD replica plate method enumerated the same sample at 3.9 logs; for this sample, we should expect the BAX®-System-SalQuant® quantification to be more accurate. These interpretations are drawn from the linear correlations that describe the strength of estimation between the two methodologies described by Vargas et al. (2023).

CONCLUSIONS

In conclusion*, Salmonella* prevalence varies by LN type in different seasons and regions. Tonsils and mesenteric LNs were associated with the greatest overall *Salmonella* prevalence, at 36% and 35%, respectively. The highest prevalence was observed in tonsils during the spring in the eastern region. The other five sample types had a prevalence of <10%, though prevalence was generally greatest in the eastern region and during the summer or fall months. It is important to mention that tonsils and mesenteric LNs are not commonly associated with ground pork contamination because they are removed from the carcass. However, the question may be raised about cheek meat contamination from the tonsils, as well as other cross-contamination risks. Peripheral LNs, such as subiliac, prescapular, superificial inguinal, and axillary LNs, have a higher likelihood of being incorporated into ground pork because of their location in fat in the carcass. Tonsils and mesenteric LNs also had the highest concentration of *Salmonella.* The median *Salmonella* values for mesenteric LNs fell below the limit of quantification, but the

median values for tonsils was 2.18 log_{10} *Salmonella* cells/sample using BAX®-System-SalQuant®. While most samples that were positive for *Salmonella* harbored less than 3 logs, there were several samples harbored greater than 3 logs. The dilution effect that occurs when a LN is incorporated into ground pork must be considered; more research may need to be conducted to understand how one LN can contamination hundreds of pounds of ground pork in a batch. The 3M™ EB Petrifilm™+XLD replica plate method and BAX®-System-SalQuant® methodologies can both be used to quantify *Salmonella* in market hog LNs.

Pork processing facilities can use these findings to create mitigation strategies designed for controlling *Salmonella* contamination in LNs, though further research may be necessary to identify the best mitigation strategies. *Salmonella* continues to plague in pork systems, prompting the study of tissues that harbor this pathogen. The results from this study reinforced data previously reported in the literature by indicating that market hog LNs harbor *Salmonella* in various lymphoid tissue. The risk of *Salmonella* contaminated LNs is greatest when the interior of a LN is exposed. Lymph nodes are also commonly associated with ground pork products, thus representing a risk for disease in humans if the products are not cooked or handled properly.

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DATA AVAILABILITY STATEMENT

Data are available from the corresponding author upon request.

CONFLICT OF INTEREST STATEMENT

The authors have no competing or financial interests associated with publication of this article.

AUTHOR CONTRIBUTION STATEMENT

E.F.: Investigation (equal), Data curation (equal), Writing—Original Draft (lead), Writing—Review & Editing (lead), Visualization (lead).

D.V.: Investigation (equal), Data curation (equal), Writing—Original Draft (supporting), Writing—Review & Editing (supporting), Visualization (supporting).

G.K.B-B.: Investigation (supporting), Data curation (supporting), Writing—Review & Editing (supporting)

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FIGURES AND TABLES

Figure 1. Market hog sampling regions represented by the west (A), central (B), and east (C).

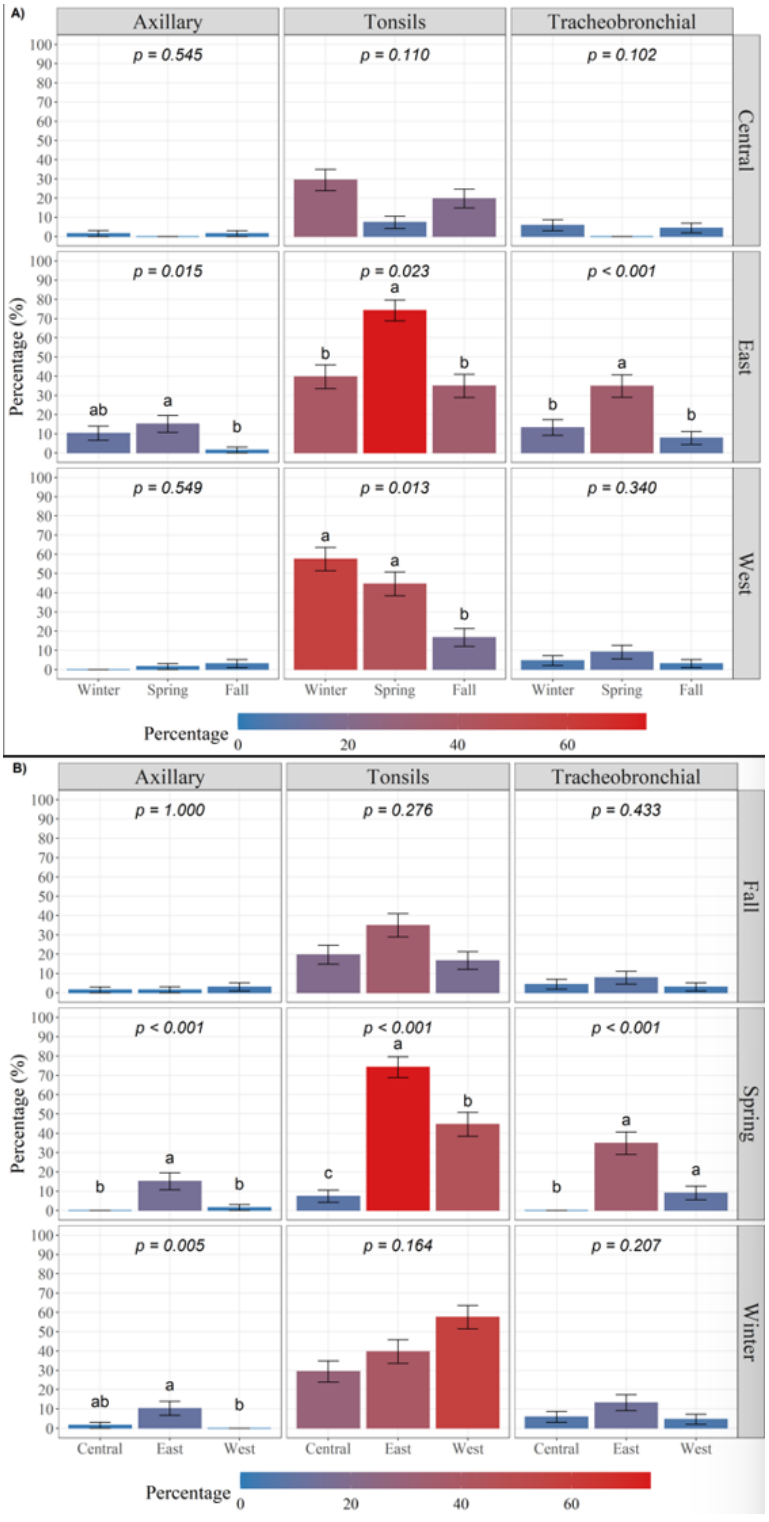


Figure 2. Graphical representation of *Salmonella* prevalence (%) by sample type with corresponding p-values where the season-by-region interaction was significant for prevalence (axillary LNs (P=0.038), tonsils (P=0.006), and tracheobronchial LNs (P=0.007)). The figure is divided by (A) *Salmonella* prevalence by region (central, east, and west) and (B) *Salmonella* prevalence by season (winter, spring, and fall) for the three significant (P<0.05) sample types.

[a,b,c] Superscripts that differ within a season and region for a specific sample type vary statistically ($P \leq 0.05$).

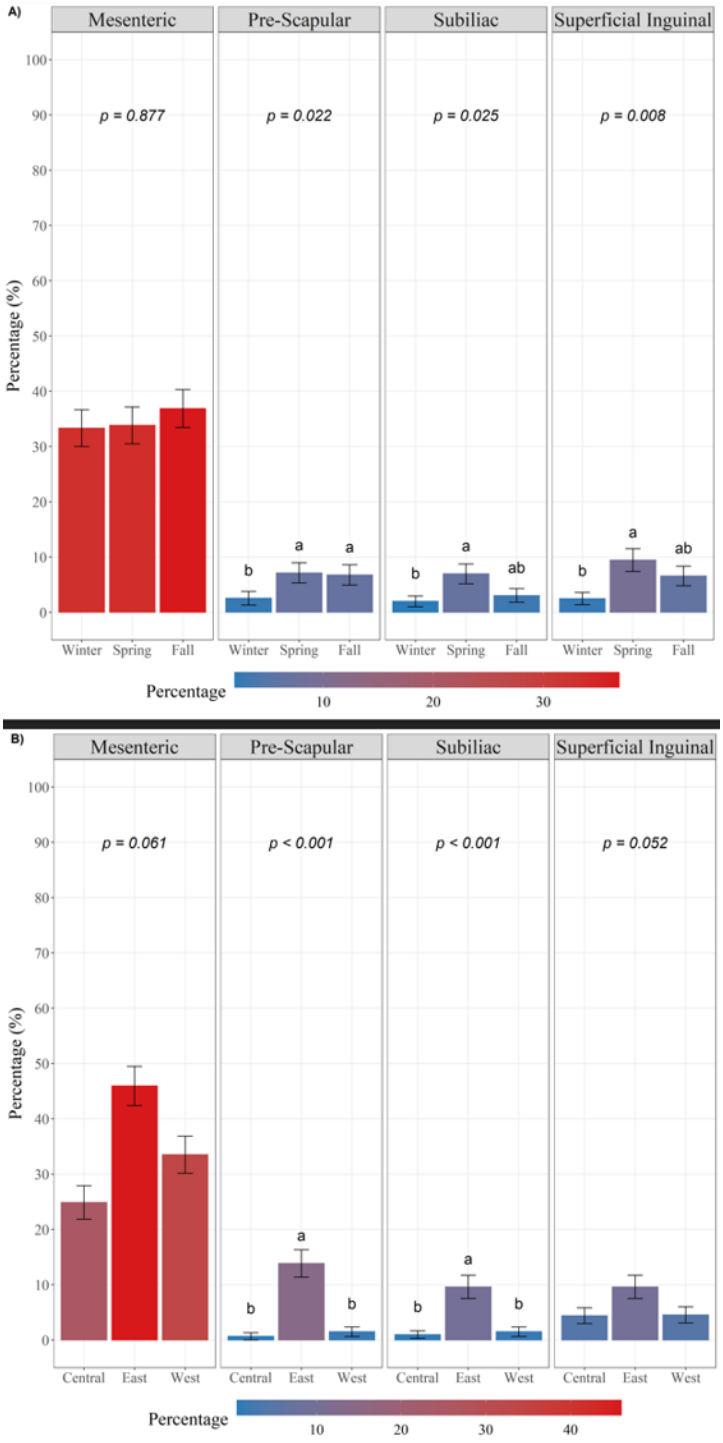


Figure 3. Graphical representation of *Salmonella* prevalence (%) by sample type with corresponding p-values where the season-by-region interaction was not significant for prevalence (mesenteric LNs (*P*=0.188), pre-scapular LNs (*P*=0.157), subiliac LNs (*P*=0.136), and superficial inguinal LNs (*P*=0.122)). The figure is divided by (A) *Salmonella* prevalence by season (winter, spring, and fall) for each sample type and (B) *Salmonella* prevalence by region for each sample type (central, east, and west) for the four sample types that were not significant (*P*>0.05) for season-by-region interaction.

[a,b,c] Superscripts that differ within a specific sample type vary statistically (P≤0.05) by season (A) or region (B).

659

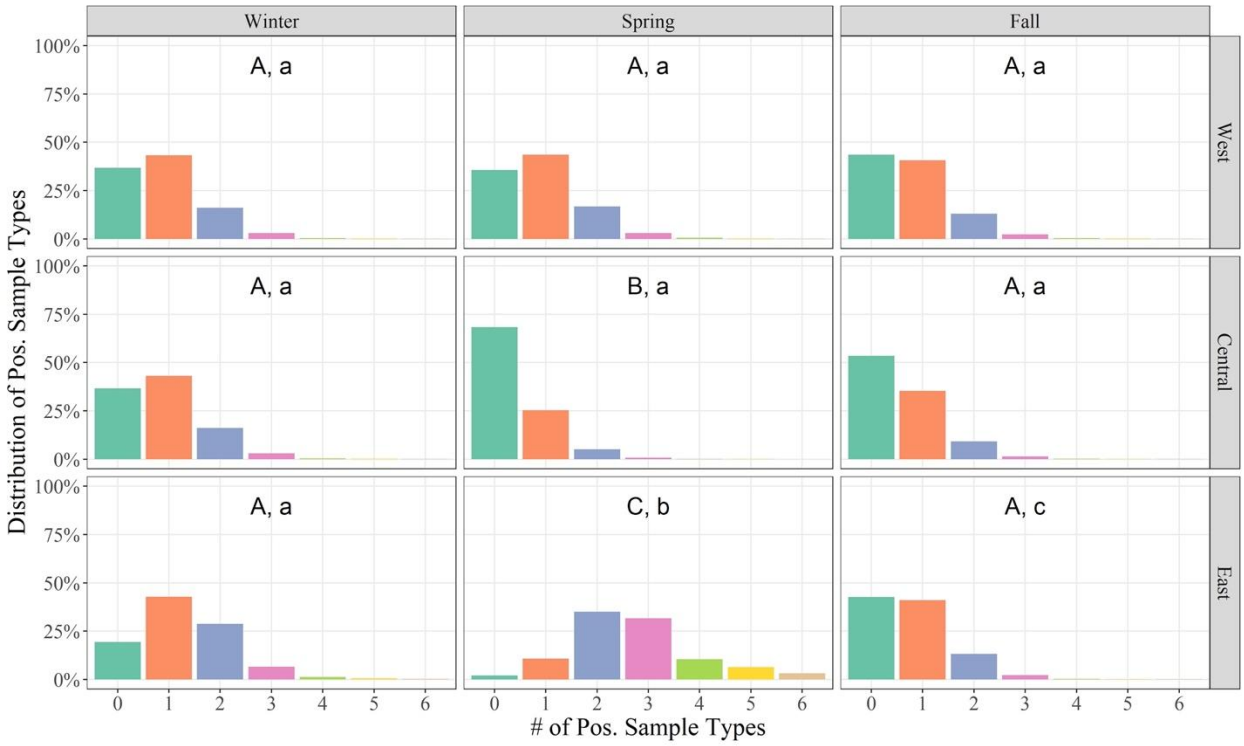


Figure 4. Model-based estimate of the distribution of the number of *Salmonella* positive sample types (lymph nodes and tonsils) per market hog carcass according to season and region.

[A,B,C] Superscripts that differ within a specific season vary statistically ($P \leq 0.05$) by region.

[a,b,c] Superscripts that differ within a specific region vary statistically ($P \leq 0.05$) by season.

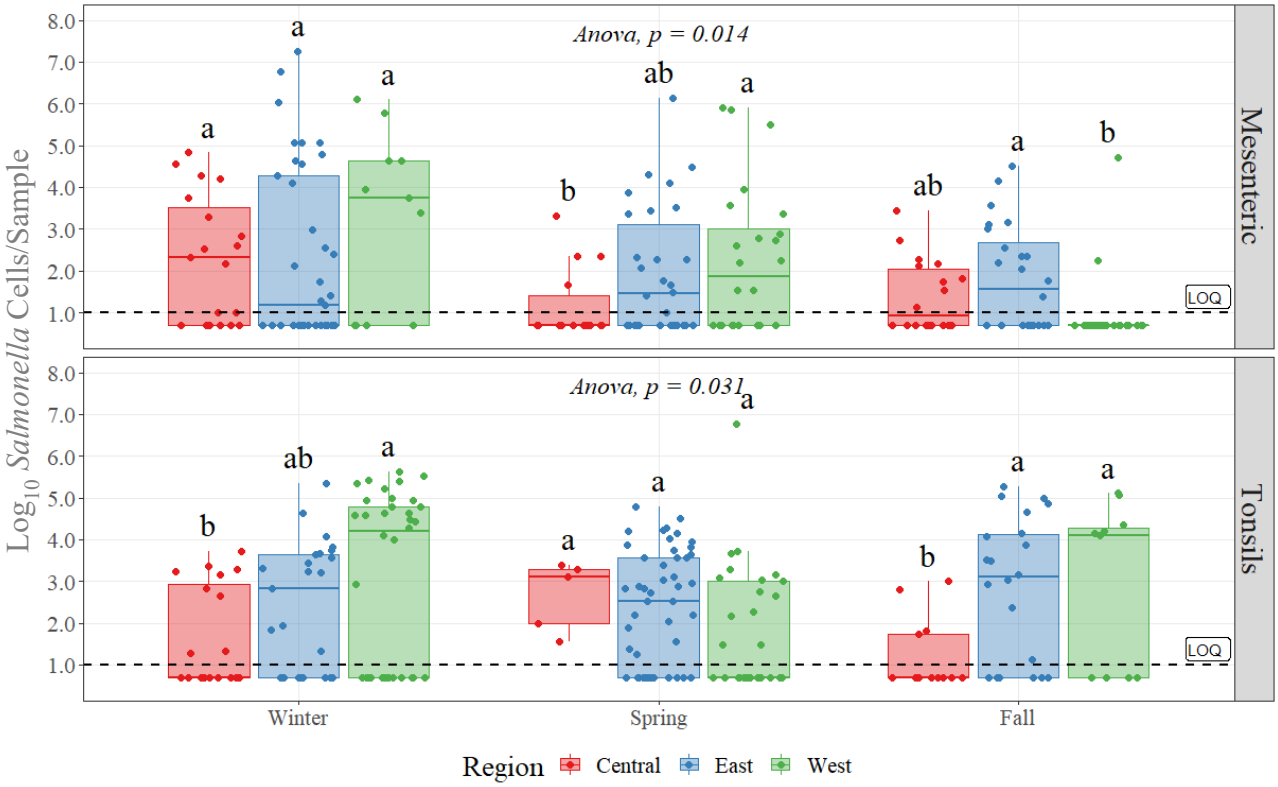
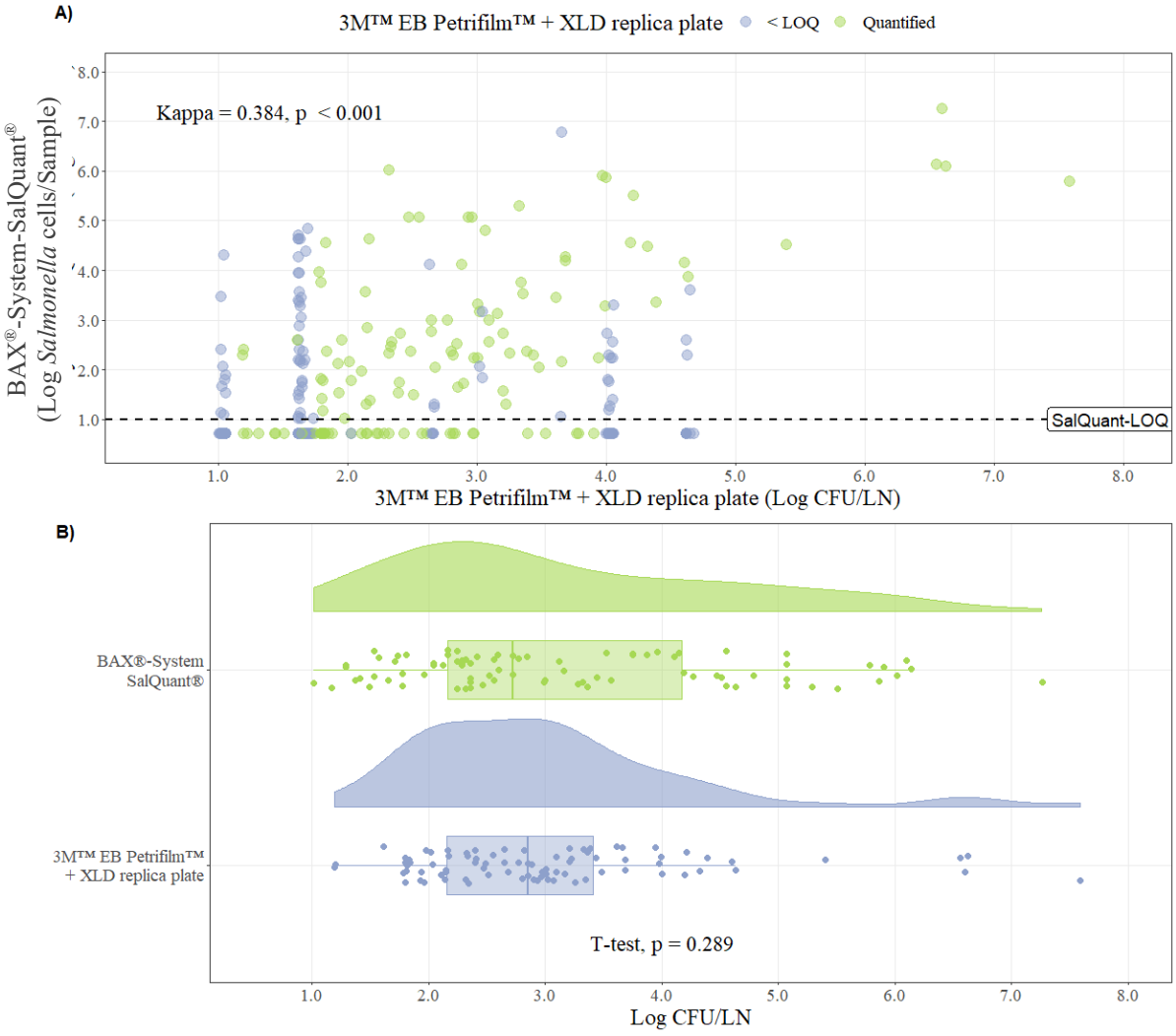


Figure 5. Bar charts of *Salmonella* $\log_{10}$ (*Salmonella* cells/Sample) concentration from ANOVA test organized by region with season for sample types where season-by-region interaction was significant (mesenteric LNs (P=0.014) and tonsils (P=0.031) and were *Salmonella* positive. Only mesenteric LNs and tonsils were statistically analyzed for *Salmonella* concentration.

[a,b,c] Superscripts that differ within a specific sample type vary statistically ($P \leq 0.05$) by season.



676

677 **Figure 6.** Graphical representation of the comparison between 3M^{TM} EB Petrifilm^{TM} + XLD
678 replicate plate and BAX®-System-SalQuant® for enumeration of *Salmonella* in pork lymph
679 nodes. A) Enumeration of *Salmonella* in pork lymph nodes using 3M^{TM} EB Petrifilm^{TM} + XLD
680 (Log CFU/Sample) replicate plate and BAX®-System-SalQuant® (Log *Salmonella* cells/Sample)
681 (n = 378 samples). The dots represent the actual data points. LOQ: Limit of Quantification. B)
682 Raincloud plot of *Salmonella* counts using 3M^{TM} EB Petrifilm^{TM} + XLD (Log CFU/Sample)
683 replicate plate and BAX®-System-SalQuant® (Log *Salmonella* cells/Sample) in pork lymph
684 nodes with counts above limit of quantification for both methodologies (n = 83 samples). In each
685 boxplot, the horizontal line crossing the box represents the median, the bottom and top box are
686 the lower and upper quartiles, the vertical top line represents 1.5 times the interquartile range,
687 and the vertical bottom line represents 1.5 times the lower interquartile range. The dots represent
688 the actual data points. Distribution plots were generated using the kernel density estimates as the
689 probability density function of a continuous variable.

690

691 **Table 1.** Explanatory variables in a model representing the level of *Salmonella* contamination in
692 a sample (detected and quantified by BAX®-System-SalQuant® were categorized into three
693 groups: ND; [LOD, LOQ); ≥LOQ.
694

24h Enrichment Detection	**6h Enrichment Quantification**	***Salmonella* Cells/Sample**
Negative	Not Available	Non-Detect (ND)
Positive	<LOQ	[1,10)
Positive	≥LOQ	Value derived from the standard curve

695
696
697

Table 2. Summary of *Salmonella* prevalence status reported by carcass in each season and region. Season by region interaction for *Salmonella* prevalence was significant for carcass (P=0.001).

Season	Region	# of Carcasses	Carcass Count	Prevalence
Winter	**West**	66	44	66.7%
	Central	68	38	55.9%
	East	68	52	76.5%
	Across	202	134	66.3%
Spring	**West**	66	43	65.2%
	Central	69	18	26.1%
	East	66	60	90.9%
	Across	201	121	60.2%
Fall	**West**	66	39	59.1%
	Central	68	35	51.5%
	East	64	41	64.1%
	Across	198	115	58.1%
All		601	370	61.6%

Table 3. Distribution of *Salmonella* in lymph nodes by market hog carcass. Tonsils are included in the lymph node count.

Number of Lymph Nodes Harboring *Salmonella*	Number of Carcasses (%)
0	38.40% (231/601)
1	36.60% (220/601)
2	17.10% (103/601)
3	5.49% (7/601)
4	1.16% (7/601)
5	0.83% (5/601)
6	0.33 (2/601)
7	0.00% (0/601)

Table 4. Summary of *Salmonella* prevalence status of cecal fluid, spleens, and ropes during the spring and fall sampling seasons according to each region.

Season	Region	Cecal Fluid		Spleens		Ropes	
		% (N)	SEM*	% (N)	SEM	% (N)	SEM
Spring	West	No Data	--	No Data	--	100 (3)	0.0
	Central	20.3 (69)	0.05	0.0 (69)	0.0	88.9 (9)	0.1
	East	36.1 (36)	0.08	26.5 (68)	0.05	No Data	--
	Across	23.5 (102)	0.04	13.1 (137)	0.03	91.7 (12)	0.08
Fall	West	71.2 (66)	0.06	4.5 (66)	0.03	No Data	--
	Central	35.3 (68)	0.06	26.3 (68)	0.03	100 (10)	0.0
	East	24.2 (33)	0.08	26.9 (65)	0.03	No Data	--
	Across	47.3 (167)	0.04	6.5 (199)	0.02	100 (10)	0.0
All		38.3 (269)	0.03	9.2 (336)	0.02	95.5 (22)	0.05

*SEM indicates standard error of the mean.

Table 5. Overall *Salmonella* prevalence, concentration, and contamination risk for market hog tonsils and lymph nodes (axillary, mesenteric, pre-scapular, subiliac, superficial inguinal, and tracheobronchial).

Sample Type	Prevalence	Median Concentration	Interpretation^	Contamination Risk
Axillary	4%	< 1 log *Salmonella* cells/Sample	Low Concentration Low Prevalence	Ground Product, Cross-Contamination
Mesenteric	35%	< 1 log *Salmonella* cells/Sample	Low Concentration, High Prevalence	Cross-Contamination
Pre-scapular	6%	< 1 log *Salmonella* cells/Sample	Low Concentration Low Prevalence	Ground Product, Cross-Contamination
Subiliac	4%	< 1 log *Salmonella* cells/Sample	Low Concentration Low Prevalence	Ground Product, Cross-Contamination
Superficial Inguinal	6%	< 1 log *Salmonella* cells/Sample	Low Concentration Low Prevalence	Ground Product, Cross-Contamination
Tracheobronchial	9%	< 1 log *Salmonella* cells/Sample	Low Concentration Low Prevalence	Ground Product, Cross-Contamination
Tonsils*	36%	2.18 log *Salmonella* cells/Sample*	Moderate Concentration High Prevalence	Cross-Contamination

* Lymph node SalQuant® protocol used to quantify tonsils and this protocol is likely not effective for tonsils.

^ Represents interpretation by the authors only.

Table 6. Contingency table of the number of *Salmonella* positive samples quantified from market hog LNs using both methodologies of 3M™ EB Petrifilm™+XLD replica plate method and BAX®-System-SalQuant®.

BAX®-System-SalQuant®	3M™ EB Petrifilm™ + XLD Replicate Plate		Total
	< LOQ	Quantified	
< LOQ	183	45	228
Quantified	67	83	150
Total	250	128	378

[1] The lower limit of quantification for BAX®-System-SalQuant® is ~10 *Salmonella* cells/sample (1 log/Sample) for small LNs, and ~50 CFU/Sample (1.7 log CFU/Sample) for medium LNs. The lower limit of quantification for 3M™ EB Petrifilm™+XLD replicate plate method is 0.5 CFU/mL (because of duplicate plating).

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