National survey of *Salmonella* prevalence in lymph nodes of sows and market hogs[1]

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ABSTRACT: Livestock are known to harbor *Salmonella* in their gastrointestinal (**GI**) tract and lymphatic tissues. Pathogens may be transferred from the GI tract to external carcass surfaces during normal harvest procedures but can be mitigated by antimicrobial carcass interventions. Lymph nodes (**LNs**) are typically encased in fat and are protected from antimicrobial carcass surface treatments, thus serving as a possible root cause of foodborne illnesses attributed to *Salmonella* in meat products. Members of the pork industry are committed to food safety and want to better understand *Salmonella* as a potential contaminant in pork products. To establish a baseline of *Salmonella* prevalence in porcine LNs across the United States, 21 commercial pork harvest facilities, representing northern (n = 12) or southern (n = 9) geographical regions, participated in this study. As processing volumes allowed, 25 carcasses were selected from each establishment. From each carcass, left and right superficial inguinal LNs (n =1,014 LNs) were removed and pooled to yield one sample per animal or n = 507 total LN samples. *Salmonella* prevalence rates differed (P < 0.05) between hog types in both regions. Specifically, 6.4% of market hog and 37.0% of sow samples were *Salmonella* positive in the northern region. This was reversed in the southern region as 13.0% of market hog and 4.8% of sow samples were *Salmonella* positive. There also was a difference (P < 0.05) in prevalence rates between northern and southern regions for sows, but not market hogs (P > 0.05). Type of chilling method (conventional, blast, or other) used at each market hog facility (n = 12) was documented. In the northern region, prevalence rates of *Salmonella* across chilling types were as follows: 20.0%, 2.7%, and 1.3% positive samples for conventional, other, and blast chill methods, respectively. In the southern region, 20.0% of samples were positive for conventional, 0.0% for blast, and 12.0% for other chilling methods. In both regions, samples from conventionally chilled carcasses returned more (P < 0.05) positive results than any other chill method. Overall, the higher rate of *Salmonella* prevalence in northern sows warrants further investigation, and members of the pork industry would benefit from the identification of possible methods to address the presence of *Salmonella* in porcine LNs.

Key words: lymph nodes, market hogs, prevalence, *Salmonella,* sows

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INTRODUCTION

According to the Centers for Disease Control and Prevention (2013), *Salmonella* in pork is the third leading cause of foodborne illness-related hospitalizations. Foodborne illness-related medical costs create an enormous financial burden, even before determining lost revenue from associated product recalls. For example, the United States Department of Agriculture – Economic Research Service (2014) estimates that the annual cost of foodborne illnesses caused by *Salmonella* in 2013 was $3.7 billion.

Salmonella has been found in pork lymph nodes (**LNs**), feces, and carcass samples (Berends et al., 1996; Hurd et al., 2001), which is particularly important when considering food safety implications for *Salmonella.* In 1996, the United States Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) implemented the Pathogen Reduction and Hazard Analysis and Critical Control Point System. This established performance standards for *Escherichia coli* and *Salmonella* (United States Department of Agriculture – Food Safety and Inspection Service, 1996). A few years later, the USDA-FSIS implemented new performance standards for *Salmonella* (United States Department of Agriculture - Food Safety and Inspection Service, 1998), and most recently, the USDA-FSIS proposed a rule that would implement a new inspection system and pathogen testing for market hog slaughter (United States Department of Agriculture - Food Safety and Inspection Service, 2018).

The more the pork industry understands about *Salmonella* contamination, the more successful it will be at controlling the pathogen. The lymphatic system is a part of the immune system and filters out bacteria and viruses for eventual destruction by the body. LNs have been identified as a source of *Salmonella,* because LNs can harbor microorganisms within them. Generally, the majority of studies regarding LNs have been conducted on LNs located in the gastrointestinal tract. Nevertheless, *Salmonella* has been identified in peripheral LNs that have the potential to be incorporated into ground pork products. *Salmonella* in peripheral LNs is an issue because they are protected from carcass interventions due to the surrounding fat tissues. The present study was designed to benchmark *Salmonella* prevalence rates in the LNs of U.S. sows and market hogs. Data from this study have the potential to influence decisions related to pre- and post-harvest interventions for reducing *Salmonella* in pork, which could in turn potentially reduce the number of salmonellosis cases attributed to pork products.

MATERIALS AND METHODS

Sample Collection

Thirty-three commercial pork harvest and processing facilities were initially identified as potential participants in this study and categorized by hog type (sow or market hog) and geographical region (northern or southern; Figure 1). A total of 21 (n = 8 northern market hog, n = 4 northern sow, n = 4 southern market hog, and n = 5 southern sow) facilities participated in the study; the remaining 12 establishments either declined or were no longer in operation. In-plant LN collections in the northern and southern regions were conducted by Penn State

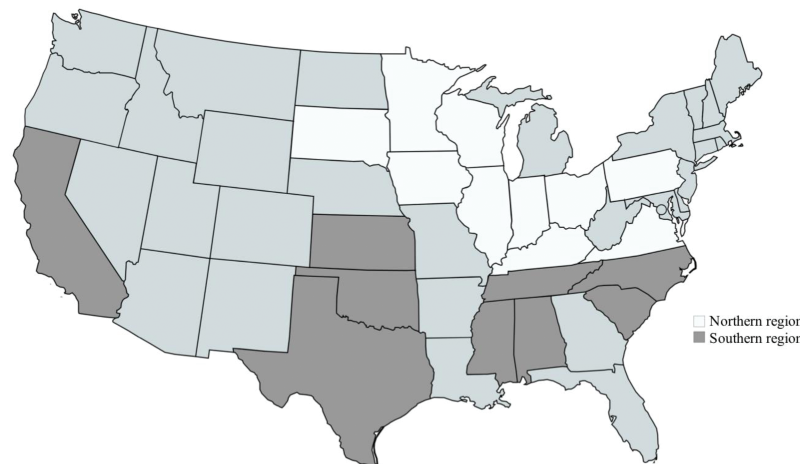

Figure 1. Map of northern and southern regions determined during the identification of 33 commercial pork harvest and processing facilities as potential participants in this study. A total of 21 (n = 8 northern market hog, n = 4 northern sow, n = 4 southern market hog, and n = 5 southern sow) facilities ultimately participated; the remaining 12 establishments either declined or were no longer in operation.

University and Texas A&M University personnel, respectively.

In addition to LN sample collection, type of carcass chilling method (conventional, blast, or other) used at each facility was documented. Carcass chilling methods were defined as follows: 1) conventional—standard cold storage unit without forced air circulation or water spray; 2) blast chill—cold storage unit with forced air circulation but without water spray; or 3) other—conventional or blast chill with water spray or another quick chill system. Carcass chilling methods were only documented for establishments harvesting market hogs, as all sow carcasses were hot-boned.

Sample Collection and Processing

Twenty-five carcasses were selected from each establishment, except for one sow facility with a low processing volume and one market hog facility where two extra carcasses were sampled. All samples were collected between December 2016 and August 2017. To minimize disruption of lean tissues and processing activities, the superficial inguinal LN was selected for use in this study. From each carcass, left and right superficial inguinal LNs (n =1,014) were removed and pooled, yielding one sample per animal or n = 507 total LN samples. Samples were sealed in sterile sample bags (VWR International, Radnor, PA), packed in insulated hard plastic coolers with refrigerant materials, and shipped via overnight carrier within 24 h of sample collection to the Animal Disease Research and Diagnostic Laboratory (ADRDL) at South Dakota State University (SDSU, Brookings, SD). Upon arrival, LNs were aseptically removed from surrounding fat tissue using flame-sterilized scalpel and forceps. De-fatted LNs were flame-sterilized to remove any surface contamination, weighed, placed into sterile filter bags (Whirl-Pak, Nasco, Sandy Springs, GA), and pulverized using a rubber mallet. Pulverized LN samples were processed immediately as described below.

Salmonella *Detection and Confirmation*

LN samples were pre-enriched with 90 mL of buffered peptone water (**BPW**; Sigma, St. Louis, MO) and incubated for 18 to 24 h at 37 °C. Entire LN samples were analyzed for the presence of *Salmonella* using the methods suggested for "raw meat and raw beef mixed products" according to the procedures outlined in the Microbiological Laboratory Guidebook 4.08—"Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and Catfish Products and Carcass and Environmental Sponges" (United States Department of Agriculture – Food Safety and Inspection Service, 2014).

Salmonella in pulverized LN samples was detected using real-time PCR method (BAX System; DuPont Qualicon, Wilmington, DE) by screening the overnight enrichment for the presence of *Salmonella* DNA. Only the samples that were either BAX PCR positive or indeterminate were used for subsequent *Salmonella* isolation steps. One milliliter of pre-enriched cultures of these samples were transferred to 10-mL Tetrathionate broth (BD Difco; Sparks, MD) and incubated at 37 °C for 18 h. After incubation, cultures were streak plated onto selective Xylose-Lysine-Tergitol 4 agar (BD Difco; Sparks, MD) and incubated overnight at 37 °C. Black colonies developed after incubation, which were presumptive positive for *Salmonella,* were further subcultured in Luria-Bertani (LB) agar plates (Fisher Sci., Hanover Park, IL) overnight at 37 °C and verified for *Salmonella enterica* using a Bruker matrix-assisted laser desorption ionization–time-of-flight mass spectrometer (Microflex LT/SH; Bruker, Bremen, Germany).

Whole Genome Sequencing (WGS) and Analysis

Of the confirmed *Salmonella*-positive samples (n = 68), establishment consent for WGS was provided for 23. Using samples from consenting establishments, isolates were grown in LB broth (Fisher Sci.) overnight at 37 °C. Following incubation, genomic DNA was isolated from 1.0 mL overnight cultures using the Qiagen DNeasy kits (Qiagen, Valencia, CA) according to manufacturer's instructions. The concentrations of genomic DNA samples were measured using Qubit Fluorometer 3.0 (Invitrogen, Carlsbad, CA) and diluted appropriately to obtain a concentration of 0.3 ng/µL for each sample. Five microliters of each sample then were processed using Nextera XT DNA Sample Prep Kit (Illumina Inc., San Diego, CA) using the manufacturer-provided protocol. Purified products with unique barcodes were normalized and equal volumes of normalized libraries were pooled together and diluted in hybridization buffer (Illumina, Inc.). WGS was performed on an Illumina Miseq platform (Illumina, Inc.) using V2 chemistry with 2 × 250 paired-end chemistry. The raw data files were demultiplexed and converted to FASTQ files using Casava v.1.8.2. (Illumina, Inc.). Serovars were

predicted from the FASTQ files using SeqSero 1.0 (Zhang et al., 2015).

Statistical Analyses

Data were analyzed using JMP Pro Software v13.1.0 (SAS Institute, Inc., Cary, NC). For *Salmonella* prevalence data, contingency tables were produced for region (northern and southern) and hog type (market hog and sow), and within-table differences were determined using Fisher's exact test and an $\alpha = 0.05$. To determine differences across chilling methods (conventional, blast chill, and other) within a given region, the Bonferroni correction for multiple tests was applied to determine significant differences between pairs using an $\alpha = 0.017$.

RESULTS AND DISCUSSION

In the present study, 13.4% of LN samples were confirmed as *Salmonella*-positive (68 positives/507 total samples). Kampelmacher et al. (1963) found 181/600 (30.1%) *Salmonella*-positive samples from a variety of porcine sources, including crura of diaphragm (5.5%), spleen (3.1%), liver (3.9%), gallbladder (9.6%), mesenteric LNs (15.0%), portal LNs (8.0%), and feces (11%). In a similar study, *Salmonella* occurred in 25.6% of rectal content samples, 19.6% of tonsils, 9.3% of mesenteric LNs, and 1.4% of carcass swabs (Swanenburg et al., 2001). Similarly, Vieira-Pinto et al. (2005) found positive samples in the ileum (13.9%), ileocolic LNs (18.8%), tonsils (9.9%), and for the mandibular LNs (12.9%). Although the aforementioned studies provide evidence that porcine peripheral and mesenteric LNs can harbor *Salmonella*, Wang et al. (2010) found 100% *Salmonella*-negative samples in a study that used PCR and cultural methods to analyze 431 porcine subiliac LNs. Furthermore, in a two-part study conducted by Bahnson et al. (2006b), *Salmonella* was not detected in any of 300 prescapular LNs analyzed in part one of the study. In the second part of the study, ileocecal LNs were collected from 10 swine herds. Of these, five tested positive for *Salmonella* (Bahnson et al., 2006b). Although these studies may not be direct comparisons to our present work, this provides additional evidence that commercial hog populations can harbor *Salmonella* in their LNs.

Data from the present study revealed regional differences in *Salmonella* prevalence (Table 1). Within each region, *Salmonella* prevalence rates between hog types differed ($P < 0.05$). *Salmonella*-positive

Table 1. Prevalence of *Salmonella*-positive LNs samples[1] by hog type and region

	Region	
	Northern	Southern
Hog type		
Market hog	6.4 (13/202) A, X	13.0 (13/100) A, X
Sow	37.0 (37/100) B, X	4.8 (5/105) B, Y

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

X,Y: Values within a row lacking a common letter differ ($P < 0.05$).

[1]A total of 21 ($n = 8$ northern market hog, $n = 4$ northern sow, $n = 4$ southern market hog, and $n = 5$ southern sow) commercial harvest and processing facilities participated in the study; the remaining 12 facilities either declined or were no longer in operation. At each commercial facility, market hogs or sows were harvested and left and right superficial inguinal LNs ($n = 1,014$ LNs) were removed. Within animal, left and right LNs of each type were pooled ($n = 507$ total samples).

sow samples (37.0%) occurred more frequently than market hog samples (6.4%) collected in the northern region, whereas in the south, a higher rate of *Salmonella* prevalence was found in market hog samples (13.0%) than from sow carcasses (4.8%). Overall, the rate of *Salmonella* prevalence was higher ($P < 0.05$) in sow samples from the northern region when compared with the southern region, whereas the rate of prevalence in market hog samples did not differ ($P > 0.05$) between regions.

In 1995, researchers (United States Department of Agriculture – Animal and Plant Health Inspection Service, 1995) gathered information on *Salmonella* prevalence from on-farm feces samples from pork producers around the United States, ultimately finding 38.2% *Salmonella*-positive samples. Furthermore, they showed 65.5%, 36.1%, and 29.9% prevalence rates for the southeastern, north central, and midwest regions, respectively (United States Department of Agriculture – Animal and Plant Health Inspection Service, 1995). O'Connor et al. (2006) conducted a two-part study on *Salmonella* antibodies by collecting diaphragm samples from low- and high-volume Iowa pork producers. Of the samples tested, 18.9% of hog lots from low-volume producers and 19.7% of hog lots from high-volume producers tested positive for *Salmonella* antibodies (O'Connor et al., 2006). Although this study evaluated *Salmonella* antibodies, it exhibits how many hogs were exposed to *Salmonella* while on farm. Percentages for antibodies from hog lots in both low- and high-volume producers in this study were slightly higher than what was observed in the present study, in which overall northern region prevalence was found to be 16.6% (data not presented in tabular form). Additionally, Bahnson et al. (2006a) investigated *S. enterica* prevalence from ileocolic

LNs of hogs in midwest swine herds. *Salmonella* was found in 100 of 146 herds sampled (68.5%), with an individual-pig prevalence rate of ~7.0% within-herd (Bahnson et al., 2006a). These data demonstrate the potential for high prevalence rates of *Salmonella* in hog herds in the northern region. In South Korea, Jung et al. (2001) found 17.9% *Salmonella*-positive LNs from the 784 ileocecal LNs. This rate of prevalence is slightly higher than findings from our study in which overall regional prevalence was 16.6% and 8.8% for northern and southern, respectively (data not presented in tabular form).

Data available on *Salmonella* prevalence within sex-type are very limited. Larsen et al. (2003) determined the prevalence of *Salmonella* in cull sows using many different tissue types, including ileocecal, ventral thoracic, and subiliac LNs. Of the 181 samples collected, 12 ileocecal, 4 ventral thoracic, and 4 subiliac LNs were positive for *Salmonella*, resulting in an overall *Salmonella*-prevalence rate of 8.8% (Larsen et al., 2003). We found overall hog-type prevalence to be 20.5% and 8.6% *Salmonella*-positive samples for sows and market hogs, respectively (data not presented in tabular form). These values are higher than the prevalence rate documented by Larsen et al. (2003).

As seen in Table 2, the rate of *Salmonella* prevalence was highest ($P < 0.017$) for the conventional chill method when compared with other chill types for samples collected in the northern region

Table 2. Prevalence of *Salmonella*-positive LNs samples[1] by chilling method[2] and region for market hogs[3]

	Region	
	Northern	Southern
Chill type		
Conventional	20.0 (10/50) $_A$	20.0 (10/50) $_A$
Blast chill	1.3 (1/77) $_B$	0.0 (0/25) $_A$
Other	2.7 (2/75) $_B$	12.0 (3/25) $_A$

A,B: Values within a column lacking a common letter differ ($P < 0.017$).

[1]A total of 21 ($n = 8$ northern market hog, $n = 4$ northern sow, $n = 4$ southern market hog, and $n = 5$ southern sow) commercial harvest and processing facilities participated in the study; the remaining 12 facilities either declined or were no longer in operation. At each commercial facility, market hogs or sows were harvested and left and right superficial inguinal LNs ($n = 1{,}014$ LNs) were removed. Within animal, left and right LNs of each type were pooled ($n = 507$ total samples).

[2]Carcass chilling methods were defined as follows: 1) conventional—standard cold storage unit without forced air circulation or water spray; 2) blast chill—cold storage unit with forced air circulation and without water spray; or 3) other—conventional or blast chill with water spray or other quick chill system.

[3]Carcass chilling methods were only documented for establishments harvesting market hogs, as all sow carcasses were hot-boned.

(conventional 20.0%; blast chill 1.3%; other 2.7%). No differences in *Salmonella* prevalence were seen between chilling methods in the southern region (conventional 20.0%; blast chill 0.0%; other 12.0%). Conventional chilling could be considered the slowest method of the three chilling styles evaluated. The implication that slower, conventional chilling could affect counts and/or detection of *Salmonella* is in agreement with previous work. Chang et al. (2003) investigated the impact of blast versus conventional chilling on survival of *Salmonella* Typhimurium inoculated onto skin-on and skin-off pork carcass surfaces. Chang et al. (2003) found that regardless of carcass surface type (skin-on or skin-off) or inoculum level used, high (5.0 log CFU/cm^2) or low (3.0 log CFU/cm^2), both blast and conventional chilling methods produced lower bacterial counts than the control. However, conventionally chilled carcasses consistently returned higher counts of *S.* Typhimurium when compared with blast-chilled tissues (Chang et al., 2003). It has also been demonstrated that *S.* Typhimurium has the ability to adapt to cold temperatures, providing greater protection against subsequent stresses (Shah et al., 2013). Based on these findings, it is plausible that the time frame associated with conventional chilling could trigger a cold adaptation response in the salmonellae.

As mentioned previously, not all confirmed *Salmonella*-positive LN samples ($n = 68$) were subjected to WGS. Serovars identified from confirmed *Salmonella*-positive LN samples for which WGS consent was granted ($n = 23$) are presented in Table 3. Of these, London (21.7%), Anatum (13.0%), Worthington (8.7%), 3,10:b:e,n,x or Benfica (8.7%), and 3,10:l,z13:1,6 (8.7%) were most commonly identified. Notably, several serovars documented in the present study were also identified in available literature. In a study by Kampelmacher et al. (1963), the five most commonly identified serovars from positive portal and mesenteric LN samples were Typhimurium, Heidelburg, Bredeney, Worthington, and Newport at rates of 37.0% (67/181), 16.6% (30/181), 9.4% (17/181), 6.1% (11/181), and 5.0% (9/181), respectively. Anatum (2.8%; 5/181) and Infantis (1.1%; 2/181) also were found by Kampelmacher et al. (1963), although less often than the present study. In a follow-up experiment conducted by Edel and Kampelmacher (1976), the most frequently isolated serovars from *Salmonella*-positive porcine LNs and fecal samples were Typhimurium (31.7%; 157/496), Give (12.9%; 64/496), Infantis (8.7%; 43/496), Stanley (7.9%; 39/496), and Derby (7.5%; 37/496); Anatum and

Table 3. *Salmonella* serovars isolated from porcine LN samples

Serovar	No. isolated	% prevalence
London	5	21.7
Anatum	3	13.0
Worthington	2	8.7
3,10:b:e,n,x or Benfica	2	8.7
3,10:l,z13:1,6 (No predicted serotype)	2	8.7
Eko	1	4.4
Infantis	1	4.4
Johannesburg	1	4.4
Ohio	1	4.4
Regent	1	4.4
Uganda	1	4.4
1,3,19:f,g:1,6 (No predicted serotype)	1	4.4
1,3,19:g,s,t:1,6	1	4.4
4:f,g:1,6	1	4.4

A total of 21 (n = 8 northern market hog, n = 4 northern sow, n = 4 southern market hog, and n = 5 southern sow) commercial harvest and processing facilities participated in the study; the remaining 12 facilities either declined or were no longer in operation. At each commercial facility, market hogs or sows were harvested and left and right superficial inguinal LNs (n = 1,014 LNs) were removed. Within animal, left and right LNs of each type were pooled (n = 507 total samples). Of the confirmed *Salmonella*-positive samples (n = 68), establishment consent for whole genome sequencing was provided for 23.

Worthington were seen less often at 1.6% (8/496) and 0.4% of isolates (2/496), respectively. In the first of two studies conducted in the Midwest, Derby (23.2%; 106/455), Typhimurium (12.1%; 55/455), and Brandenburg (10.5%; 48/455) were the leading serovars identified from *Salmonella*-positive ileocolic LNs (Bahnson et al., 2006a). Bahnson et al. (2006a) also found Uganda (5.7%; 26/455) at a frequency similar to our current study, whereas London (5.0%; 23/455), Anatum (4.8%; 22/455), and Worthington (2.4%; 11/455) were identified less frequently. Common serovars were not identified when comparing our work with the second study conducted by Bahnson et al. (2006b) in which 15 *Salmonella*-positive prescapular and ileocecal LNs were analyzed; serovars were Derby (33.3%), Typhimurium (26.7%), Java (13.3%), Hartford (6.7%), Mbandaka (6.7%), and Senftenberg (6.7%). In South Korea, positive porcine LN samples were found to contain serovars Typhimurium (5.2%; 41/784), Schwarzengrund (2.9%; 23/784), Derby (2.6%; 20/784), Mbandaka (2.4%; 19/784), and Enteritidis (0.8%; 6/784) (Jung et al., 2001). Lastly, Vieira-Pinto et al. (2005) most commonly identified Typhimurium (56.3%; 18/32), Rissen (12.5%; 4/32), Tennessee (6.3%; 2/32), Enteritidis (6.3%; 2/32), Anatum (6.3%; 2/32), Give (3.1%; 1/32), and Derby (3.1%; 1/32). Overall, Typhimurium and Derby seemed to occur most often in the literature, and although neither were identified in the present study, common serovars between this and other studies include Anatum, Worthington, Infantis, London, and Uganda.

Findings from this study highlight a need for additional research to identify specific production practices contributing to *Salmonella* in LNs. Specifically, items for consideration, by both researchers and pork producers and processors, include development and implementation of on-farm production practices, veterinary treatments, and pre-harvest interventions. Additionally, developing and implementing post-harvest processing methods to reduce *Salmonella* in porcine LNs should be explored more thoroughly. Because LNs are a possible source of *Salmonella* contamination, pre-harvest practices and/or procedures for removing LNs during processing may be beneficial in reducing *Salmonella* in pork, thereby reducing foodborne illnesses.

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