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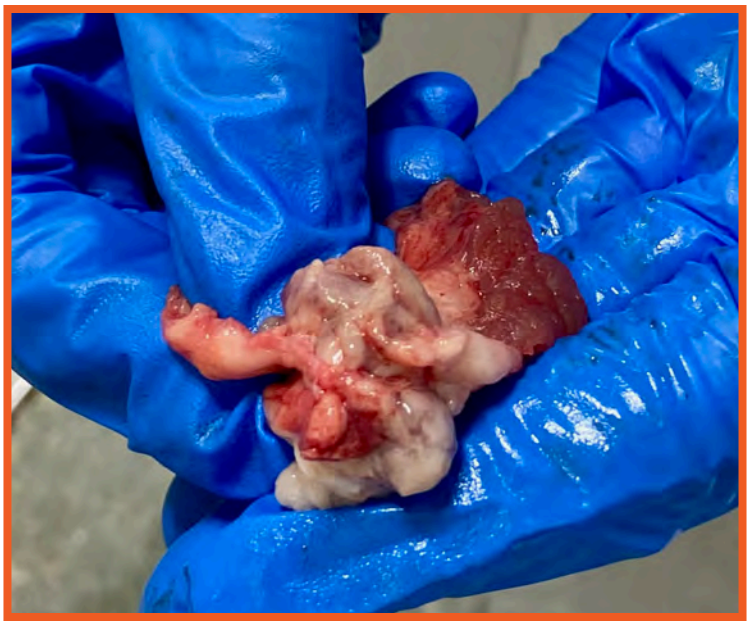

Salmonella is Present in Multiple Lymph Nodes of Market Hog Carcasses at Slaughter

ABSTRACT

The presence of *Salmonella* in lymphoid tissue of market hogs represents a potential risk for the safety of pork products, particularly ground pork. With increased *Salmonella* testing standards required by the U.S. Department of Agriculture (USDA) on the horizon for the pork industry, it is important to improve understanding of *Salmonella* contamination within a variety of swine tissues, including lymph nodes. This study was designed to provide preliminary information about *Salmonella* prevalence in multiple lymph nodes within market hog carcasses at slaughter. From each carcass (n = 50), four lymph nodes were aseptically collected at slaughter: mesenteric, tracheobronchial, inguinal, and subiliac. A total of 197 lymph nodes were analyzed, with an overall *Salmonella* prevalence of 21.8% (n = 43). *Salmonella* was detected in the lymph nodes of 62% (n = 31) of carcasses, with 21.6% (n = 11) of carcasses harboring *Salmonella* in two or more lymph node types. Although not statistically significant (P = 0.1167), *Salmonella* prevalence did vary based upon lymph node type (mesenteric, 34%; inguinal, 18.4%; subiliac, 18.4%; tracheobronchial, 16.3%). This lymph node mapping study provides preliminary evidence that *Salmonella* can contaminate lymph nodes throughout swine and may serve as the foundation for larger lymph node mapping studies or intervention strategies at the abattoir.

INTRODUCTION

Salmonella continues to be an important public health concern because salmonellosis causes an estimated 1.35 million illnesses, leads to 26,500 hospitalizations, and results in 420 deaths each year in the United States *(6)*. Salmonellosis is a disease caused by a variety of the over 2,500 identified *Salmonella* serotypes, many of which differ in their range of infection and disease-causing capabilities *(16)*. It is estimated that salmonellosis cost over $4.1 billion in the United States in 2018 *(17)*. Between 1990 and 2005, *Salmonella* was the causative agent in 25% of the pork-related foodborne outbreaks in the United States *(1)*; and risk of a *Salmonella*

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infection from eating pork, during a 40-year span of time, is estimated at 1 in 100 when considering per person-year, consuming the average number of servings or kilograms *(12)*. More recently, the Foodborne Disease Outbreak Surveillance System *(8)* reported a total of 43 outbreaks, 1,539 illnesses, 206 hospitalizations, and 3 deaths associated with *Salmonella* from pork between the years 2009 and 2015. In the European Union, pork has been recognized as the third most common cause of salmonellosis infections, behind eggs and poultry meat *(2)*.

A variety of pork products have been associated with *Salmonella* contamination; however, prevalence rates differ between ground pork and pork chops *(3)*. These differences are likely due to the compilation of muscle and adipose tissue from many animals during grinding, contamination during grinding procedures, and incorporation of lymph nodes during ground pork production *(23)*. The literature suggests that contamination of ground pork or other comminuted pork products with *Salmonella* may be due to contaminated lymph nodes *(4, 23)* due to the location of some peripheral lymph nodes (also referred to as deep-tissue lymph nodes; DTLNs) within the adipose tissue that may be incorporated into ground pork *(4)*. In addition to ground pork, contaminated lymph nodes may also cross-contaminate carcasses and primal cuts if they are cut open during slaughter *(5, 7, 15)* or fabrication.

The U.S. Department of Agriculture Food Safety Inspection Service (FSIS) commenced exploratory sampling and testing for *Salmonella* in raw pork cuts, both intact and nonintact, as well as in raw comminuted pork products in fiscal year 2019 *(18)*. Research aimed at understanding the distribution of *Salmonella* in lymph nodes located throughout market hog carcasses is necessary to support FSIS testing priorities and the pork industry and to inform future intervention strategies (e.g., lymph node removal) for *Salmonella* control. The primary objective of this study was to address these knowledge gaps by mapping the *Salmonella* prevalence in splanchnic (mesenteric and tracheobronchial) and somatic (subiliac and inguinal) lymph nodes of market hog carcasses.

MATERIALS AND METHODS

Sample collection

All lymph node samples were collected post mortem at a commercial swine processing facility in the midwestern United States. On two different sampling days (7 and 21 October 2019), four lymph nodes were collected per carcass from a total of 25 carcasses, resulting in approximately 100 lymph node samples collected per sampling day (4 lymph nodes per carcass × 25 carcasses = 100 lymph nodes). A convenience sample of 197 lymph nodes were collected in total (three lymph node samples were unable to be collected). Carcasses were removed from the processing line, and four lymph node samples were collected from each carcass, including tracheobronchial, mesenteric, subiliac, and inguinal. Relevant lot information and general observations were recorded for each carcass. Lymph node samples were placed in sterile Whirl-Pak bags (Nasco, Madison, WI) and transported to the laboratory at Kansas State University. All samples were stored at 4°C until processing, which was completed within 24 h after collection.

Sample processing

Lymph nodes were processed as described by Chaves et al. *(7)*. Briefly, fat and fascia were removed, and the lymph nodes were weighed, surface sterilized in boiling water for approximately 5 s, placed individually in filter Whirl-Pak bags (Nasco), and pulverized with a rubber mallet. All samples were then enriched with 80 ml of tryptic soy broth (TSB; BD Difco, Franklin Lakes, NJ), stomached for 1 min (Stomacher 400 Circulator, Seward, Bohemia, NY), and incubated for 6 h at 42°C. Following incubation, samples were held at 4°C for ≤12 h until subsequent analysis.

Detection of *Salmonella*

Following incubation, enrichments were subjected to automatic immunomagnetic separation as previously described by Chaves et al. *(7)* using anti-*Salmonella* Dynabeads (Applied Biosystems, Waltham, MA) and a KingFisher mL Purification System (Thermo Fisher Scientific, Waltham, MA) according to manufacturer's guidelines. Recovered anti-*Salmonella* beads were transferred to 3 ml of Rappaport-Vassiliadis broth (Oxoid, Lenexa, KS) and incubated at 35°C for 18 h. Samples were held at 4°C for ≤12 h until subsequent analysis. Following incubation, the Rappaport-Vassiliadis broth was streaked on xylose lysine desoxycholate (XLD; Remel, Lenexa, KS) and brilliant green sulfa (Remel) agar, and then incubated for 18 to 24 h at 35°C. Following incubation, XLD plates were considered presumptive positive if colonies appeared black on a pink background; brilliant green sulfa plates were considered presumptive positive if colonies appeared light pink. Presumptive positive colonies were subjected to latex agglutination (Wellcolex Colour *Salmonella*, Remel) and then streaked on XLD to isolate individual *Salmonella* colonies. XLD plates were incubated for 18 to 24 h at 37°C, and one isolated colony from each plate was then transferred to 9 ml of TSB. TSB tubes were incubated at 37°C for 24 h and then frozen in 1-ml aliquots with 10% glycerol (Thermo Fisher Scientific) at −80°C for subsequent PCR confirmation.

Salmonella confirmation

Frozen isolates were resuspended in 9 ml of TSB, incubated at 35°C for 18 to 24 h, and then subjected to PCR to confirm the presence of *Salmonella* using MicroSEQ *Salmonella* spp. kit (Applied Biosystems) with RapidFinder Express software (Applied Biosystems) and a calibrated ABI 7500 Fast Real-Time PCR machine (Applied Biosystems).

Statistical analysis

Prevalence, standard error, and confidence intervals were calculated using GraphPad Prism 9.0 (GraphPad Software,

TABLE 1. *Salmonella* prevalence (mean %) and standard error of splanchnic (mesenteric and tracheobronchial) and somatic (inguinal and subiliac) lymph nodes collected from market hogs at a midwestern U.S. facility[a]

	Lymph node category		
	Splanchnic	Somatic	Total
No. tested	99	98	197
Mean % (SE)	25.3 (4.4)[a]	18.4 (3.9)[a]	21.8 (3.0)
95% CI	16.5–34.0	10.6–26.2	16.0–27.7

[a]Indicates means do not differ significantly ($P \leq 0.05$).

TABLE 2. *Salmonella* prevalence (mean %) and standard error of mesenteric, tracheobronchial, inguinal, and subiliac lymph nodes collected from market hog carcasses at a midwestern U.S. facility[a]

	Lymph node type			
	Mesenteric	Tracheobronchial	Inguinal	Subiliac
No. tested	50	49	49	49
Mean % (SE)	34.0 (6.8)[a]	16.3 (5.3)[a]	20.4 (5.6)[a]	18.4 (5.6)[a]
95% CI	20.0–47.6	5.6–27.1	7.1–29.6	7.1–29.6

[a]Indicates means do not differ significantly ($P \leq 0.05$).

TABLE 3. Distribution of *Salmonella* in the lymph nodes of market hog carcasses (*n* = 50) from a midwestern U.S. facility

No. of lymph nodes harboring *Salmonella*	No. of carcasses* (%)
4	0 (0%)
3	1 (2.0%)
2	10 (20.0%)
1	20 (40.0%)
0	19 (38.0%)

* NOTE: 2 carcasses did not have a complete set of four lymph nodes collected.

Inc., San Diego, CA). Data were recorded and analyzed as a binomial response distribution using the chi-square test of Statistix 10 (Analytical Software, Tallahassee, FL). All *P*-values were evaluated for significance at a threshold of $P < 0.05$.

RESULTS

A total of 197 lymph nodes were analyzed, and *Salmonella* was detected in 43 samples, for an overall prevalence rate of 21.8% *(Table 1)*. *Salmonella* was detected in 25.3 and 18.4% of lymph nodes associated with the abdomen and internal organs (splanchnic, including mesenteric and tracheobronchial) and muscle tissue (somatic, including inguinal and subiliac), respectively *(Table 1)*. The difference in prevalence observed for splanchnic and somatic lymph nodes was not significant ($P = 0.2421$). When considering individual lymph node types, *Salmonella* prevalence did vary, with mesenteric lymph nodes representing the largest prevalence at 34% *(Table 2)*; however, the differences observed were not statistically significant ($P =$

TABLE 4. Number of market hog carcasses from a midwestern U.S. facility harboring *Salmonella* in splanchnic and somatic lymph nodes

Lymph node categories harboring *Salmonella*	No. of carcasses* (%)
Splanchnic	21 (42%)
Somatic	16 (32%)
Splanchnic and somatic	6 (12.0%)

*NOTE: 2 carcasses did not have a complete set of 4 lymph nodes collected.

TABLE 5. Number of lymph nodes harboring *Salmonella* in each lot of market hog carcasses sampled at a midwestern U.S. facility

Market hog carcass lot (no. of lymph nodes sampled)	No. of lymph nodes harboring *Salmonella* (%)
A (4)	0 (0%)
B (4)	2 (50.0%)
C (4)	2 (50.0%)
D (4)	2 (25.0%)
E (8)	2 (25.0%)
F (16)	2 (12.5%)
G (4)	0 (0.0%)
H (4)	1 (25.0%)
I (4)	2 (50.0%)
J (7)	0 (0.0%)
K (8)	1 (12.5%)
L (16)	3 (18.75%)
M (4)	2 (50.0%)
N (4)	1 (25.0%)
O (4)	1 (25.0%)
P (16)	6 (37.5%)
Q (12)	1 (8.3%)
R (4)	1 (25.0%)
S (4)	0 (0.0%)
T (8)	1 (12.5%)
U (20)	3 (15.0%)
V (28)	8 (28.6%)
W (10)	3 (30.0%)

0.1167). In a comparison of mesenteric lymph nodes (34%; $n = 50$) with all other lymph nodes (17.7%; $n = 147$), the mesenteric lymph nodes were significantly ($P = 0.0159$) more contaminated with *Salmonella*.

Of the 50 carcasses sampled, *Salmonella* was detected in one or more lymph nodes of 62% of carcasses, with 21.6% of carcasses harboring *Salmonella* in two or more lymph node types (*Table 3*). Zero carcasses harbored *Salmonella* in all four lymph node types sampled. When categorized as splanchnic and somatic lymph nodes, *Salmonella* was detected in 42 and 32% of carcasses, respectively, with 12% of carcasses positive for *Salmonella* in both splanchnic and somatic lymph nodes (*Table 4*). Market hog carcasses were pulled from the rail at random to represent many lots (truckloads) during each day

of sampling, and a total of 23 lots were included in this study *(Table 5)*. *Salmonella* prevalence in lymph nodes was variable across lot, ranging from 0 to 50%. Because carcasses were randomly collected, the number of lymph nodes collected per lot was also variable, ranging from 4 to 28 lymph nodes collected from carcasses within a single lot.

DISCUSSION

The goal of sampling splanchnic lymph nodes (mesenteric and tracheobronchial) was to understand *Salmonella* contamination associated with the viscera or internal organs, especially those areas located in the abdomen. Somatic lymph nodes (inguinal and subiliac) were also sampled because they are associated with muscles that may contaminate the final product if incorporated into trim products or remaining in the whole cut of muscle. The data presented herein demonstrate that *Salmonella* can be found in more than one lymph node type, including multiple lymph nodes within an individual market hog carcass. Somatic lymph nodes, which represent a more direct risk for final product contamination, were contaminated with *Salmonella*.

The overall prevalence of *Salmonella* in market hog lymph nodes was 21.8%. This prevalence is consistent with research by Vieira-Pinto et al. *(19)* that reported a prevalence of 27.7% in lymph nodes and related organs (ileum, ileocolic, tonsils, and mandibular lymph nodes). *Salmonella* prevalence was highest for mesenteric lymph nodes (34%), which is similar to the 44.4 and 20% reported by Chaves et al. *(7)* for mesenteric lymph nodes at two swine harvest facilities in Mexico. Chaves et al. *(7)* also reported that mesenteric lymph nodes had a higher *Salmonella* prevalence than subiliac lymph nodes (10.2%) and tonsils (18 and 40%). Contamination in the mesenteric lymph nodes of swine suggests asymptomatic infection, which may be the result of a recent contamination event and/or repeated infection, particularly from on-farm exposure *(5, 7, 14, 15)*.

Bessire et al. *(4)* conducted a national survey of *Salmonella* contamination in superficial inguinal lymph nodes of market hogs and sows at 21 facilities across the northern and southern United States. The authors reported a significant difference ($P < 0.05$) in prevalence of *Salmonella* in market hogs and sows in each region, with market hogs and sows, respectively, harboring 6.4 and 37.0% in the north compared to 13.0 and 4.8% in the south *(4)*. Hurd et al. *(13)* reported differences in *Salmonella* prevalence among different lymph nodes, with ileocecal (43.6%) lymph nodes having a markedly higher prevalence than subiliac (0.4%) and ventral thoracic (0.4%) lymph nodes. In comparison to Hurd et al. *(13)*, the present study also investigated subiliac lymph nodes and reported a higher prevalence of 18.4%. This variability in prevalence may be due to differences in regionality or seasonality, two variables that have not been thoroughly investigated for swine lymph nodes. The impact of region and season has been demonstrated for cattle lymph nodes *(10, 21)*; however, this has not been explored for swine lymph nodes to the same extent. Bessire et al. *(4)* demonstrated regionality, whereas Hurd et al. *(13)* demonstrated variability across different lymph node types. Additional research is necessary to understand the distribution of *Salmonella* in a variety of lymph node types and categories, at multiple facilities across regions of the United States, and during several seasons.

The pork industry has long recognized preharvest and postharvest *Salmonella* contamination of market hogs as a concern *(3, 22)*. Previous research has established that swine intestinal tracts and lymph nodes can harbor *Salmonella (3)*, which increases the risk for a *Salmonella*-positive carcass during slaughter. This study provides additional evidence for *Salmonella* contamination in swine lymph nodes. *Salmonella* contamination within lymph nodes may directly contaminate the carcass or primal cuts if the lymph node does not remain intact, thereby releasing the internal contamination during slaughter *(5, 7, 15)* or fabrication. A large body of evidence documents that lymph node contamination cannot be reduced or eliminated using traditional carcass antimicrobial intervention approaches *(10, 11, 20, 21)*; physical removal of lymph nodes during harvest or fabrication is required. Notably, the results from a quantitative microbial risk assessment conducted by Zhang et al. *(23)* suggest that improving cooking behaviors of consumers, or carcass decontamination procedures at the abattoir, may be more important than DTLN interventions (e.g., DTLN removal or interventions to reduce *Salmonella* in DTLNs) for decreasing the number of salmonellosis infections attributed to ground pork. Regardless, lymph node mapping studies such as the one described herein close the knowledge gap surrounding *Salmonella* carriage in market hog carcasses, providing processors with data to support their decision-making processes.

Many factors can enhance or contribute to the contamination levels of hogs between the farm and processing line, including transport, holding pens, stress, handling, and association with incoming pigs. Between loading and processing at the respective facilities there are many new sources that can contribute to prevalence levels: truck or trailer contamination, lairage contamination, or swine to swine contamination, to name a few. Environmental factors such as stocking density, temperature, and feed withdrawal contribute to the risk of contamination *(2)*. Hurd et al. *(13)* observed similar results, reporting that pigs can become infected from exposure to low levels of *Salmonella* Typhimurium during holding or resting periods of the preslaughter stage. Fedorka-Cray et al. *(9)* reported that contamination of mesenteric lymph nodes occurs rapidly, with the ileocolic lymph nodes of esophagotomized swine positive for *Salmonella* at 6 h following intranasal inoculation with *Salmonella* Typhimurium. This information can be used to support mitigation strategies on-farm, during transportation, and in lairage to reduce the risk for *Salmonella* contamination, including in the lymph nodes, prior to harvest. In the present study, a truckload of market hogs arriving at the

abattoir represented a lot, and *Salmonella* prevalence varied across the 23 lots sampled. Based upon the literature, it is not unreasonable to hypothesize that lymph node contamination may have occurred during transportation and preslaughter stages; however, exploring the impact of lot (i.e., truckload) on *Salmonella* prevalence in market hog lymph nodes was beyond the scope of this study and should be considered in future studies.

CONCLUSIONS

This lymph node mapping study provides an initial understanding of how *Salmonella* is distributed throughout market hog lymph nodes. The data presented herein demonstrate that multiple lymph nodes may be contaminated with *Salmonella* in market hog carcasses, some of which may contaminate final pork products. The pork industry can use these data to inform decisions regarding sampling plans, mitigation strategies, and tissues evaluation. For example, some processors may choose to sample lymph nodes for *Salmonella* contamination, whereas other processors may choose to trim some lymph nodes from carcasses. This study also has several limitations that must be considered, including a small sample size overall, sample collection from a single abattoir on only two occasions during one season, and only four lymph node types. Future research efforts can expand upon this study by investigating *Salmonella* prevalence from additional lymph nodes collected from multiple processing facilities across different regions and during different seasons. Enumerating *Salmonella* populations from a variety of market hog lymph node types and categories should also be incorporated in subsequent studies. This study, and subsequent studies, contribute to a growing body of knowledge regarding *Salmonella* in pork and aid in foodborne pathogen surveillance for the pork industry.

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