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High Prevalence of *Salmonella* in Lymph Nodes and Tonsils of Swine Presented for Slaughter in Mexico

ABSTRACT

This study reports the prevalence of culture-positive *Salmonella* in lymphoid tissues of swine presented for slaughter in two municipal abattoirs in Mexico. Fifty tonsils, and 110 mandibular, 90 mesenteric, and 115 subiliac lymph nodes (LNs), were recovered from 115 pork carcasses sampled across four days during a six-month period in a Merida harvest facility. Additionally, 10 mandibular LNs, 10 subiliac LNs, and 10 tonsils were recovered from 10 pork carcasses in a Cancun facility. The prevalence of *Salmonella* in the Merida facility was 18.0 (9/50), 12.7 (14/110), 44.4 (40/90), and 10.2% (12/115) for tonsils, mandibular, mesenteric, and subiliac LNs, respectively. In the Cancun abattoir, the prevalence was 40.0 (4/10), 20.0 (2/10), and 20.0% (2/10) for tonsils, mandibular, and mesenteric LNs, respectively. In Merida, values varied significantly across sampling days for all three LN types. These results verify that swine carry *Salmonella* systemically, posing a potential risk of cross-contamination of pork products for human consumption into which lymphoid tissues may be incorporated.

INTRODUCTION

Salmonella continues to be a leading cause of human gastroenteritis around the globe *(11)*. Swine may be symptomatic or asymptomatic carriers of *Salmonella* in the lymphatic system and in the gastrointestinal tract, which can then become an important source of contamination during transportation, finishing, lairage, slaughter, and further processing *(4, 8, 9, 12)*. Recently, Mann et al. *(14)* reported on the high diversity of viable bacteria isolated from asymptomatic and pathologically altered (hyperplastic, purulent, or granulomatous) ileocecal lymph nodes (LNs) from pigs at slaughter in Austria. From 16 LNs, the researchers obtained 209 bacterial isolates, 68% of which belonged to *Proteobacteria*, 27% to *Firmicutes*, and 5% to *Actinobacteria*. Purulent and granulomatous LNs generally contained more *Proteobacteria* than did asymptomatic and enlarged LNs. The isolates could

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be assigned to 17 bacterial genera, including Acinetobacter, *Carnobacterium*, *Escherichia*, *Lactobacillus*, and *Staphylococcus (14)*. The two most abundant pathogenic species identified were *Salmonella enterica* and *Streptococcus suis,* indicating that both commensal and pathogenic bacteria can be present in swine LNs *(14)*.

Salmonella bacteria are capable of translocating across the intestinal epithelium to underlying follicles and mesenteric lymph nodes, from which they can escape and cause systemic infections in the host *(7, 19)*. The hazard from *Salmonella*-positive lymphoid tissues is that they may be split and opened up during the slaughter process and handling, leading to direct contamination of pork carcasses and primal cuts *(4, 14)*. Furthermore, tonsils and some types of LNs remain on the pork carcass after slaughter and, along with head muscles and other tissues, may be incorporated into ground meat, sausage-type products, or mechanically separated meat *(4)*. If *Salmonella* bacteria are present in these ingredients, they may be directly responsible for contamination of final products *(4)*.

To contribute to our understanding of *Salmonella* sources and possible routes of transmission, this study was performed to estimate the prevalence of culture-positive *Salmonella* in tonsils and mandibular, mesenteric, and subiliac lymph nodes of swine presented for slaughter at two municipal (non-federally inspected) harvest facilities on the Yucatan peninsula of Mexico.

MATERIALS AND METHODS

Location and sample collection

Two municipal (non-federally inspected) mixed beef/swine abattoirs were visited, one located in Merida (Yucatan State) and the other in Cancun (Quitana Roo State). In the Merida facility, samples were collected on four different days during a six-month period (November through May). On the first two days of sampling, 30 animals per day were tagged and followed along the production line, and one mandibular, one mesenteric, and one subiliac LN were collected from each carcass. On the third day, one tonsil, one mandibular LN, and one subiliac LN were recovered from each of 25 pork carcasses. On the last sampling day, one tonsil, and one mandibular, one mesenteric, and one subiliac LN, were obtained from each of 30 pork carcasses. On the last day, one or more types of lymphoid tissue (LN or tonsil) could not be collected from 12 of the 30 pork carcasses sampled. Consequently, a total of 110 mandibular LNs, 90 mesenteric LNs, 115 subiliac LNs, and 50 tonsils were recovered from the 115 pork carcasses sampled at the Merida establishment. In the Cancun plant, samples were collected only once because of the extremely low number of animals presented for slaughter and lack of production at the facility. Ten mandibular LNs, 10 subiliac LNs, and 10 tonsils were recovered from 10 pork carcasses in this facility. According to the general managers of both plants, swine are brought to the facilities from local farms. However, no records existed on how many farms supply animals for slaughter on any given day, the health status of the animals, or any other pertinent animal production information.

Sample processing

Each LN, along with its surrounding fat and/or connective tissue, was placed in a sterile Whirl Pak® bag (Nasco, Jackson, WI) and transported back to the Texas Tech University Food Safety Laboratories in insulated containers with coolant packs. USDA Animal and Plant Health Inspection Service (APHIS) permits (permit number 114031) to bring samples back to the U.S. were obtained in advance. All porcine material was subjected to 70% ethanol prior to entry into the United States. Upon arrival to the laboratory, samples were immediately stored in refrigeration at 4°C until microbiological analyses were performed within 48 h of sample collection.

Detection of *Salmonella*

All LNs were processed and analyzed for the presence of *Salmonella* as previously described *(3)*. Briefly, surrounding fat and fascia were carefully trimmed, and the LNs were surface-sterilized in a boiling water bath. Each LN was placed in an individual filter sample bag (Nasco, Jackson, WI), pulverized with a rubber mallet, enriched in 80 ml of tryptic soy broth (TSB; EMD, Darmstadt, Germany), and incubated for 6 h at 42°C *(10)*. Sample enrichments were subjected to immunomagnetic separation (IMS) using anti-*Salmonella* beads (Dynabeads; Invitrogen, Carlsbad, CA). Recovered IMS beads were transferred to Rappaport-Vassiliadis (RV; Remel, St. Louis, MO) broth, incubated at 35°C for 18 hours, and streaked onto xylose lysine deoxycholate (XLD; Remel, St. Louis, MO) and brilliant green sulfa (BGS) agar plates. Typical *Salmonella* isolates (colonies with black centers on XLD and pink colonies on BGS) were subjected to latex agglutination (Remel, Lenexa, KS). Colonies presumptively confirmed as *Salmonella* via latex agglutination were transferred to 9 ml of TSB containing 10% glycerol (EMD, Darmstadt, Germany) prior to incubation at 37°C for 18 to 24 h. From each isolate tube, 1-ml aliquots were frozen in duplicate at -80°C for future use and further characterization.

Statistical analyses

Data were analyzed as a binomial response distribution to estimate the prevalence of *Salmonella* in tonsils and in the different types of LNs. The Statistical Analysis Software SAS, version 9.4; SAS Institute, Cary, NC, was used to estimate 95% confidence intervals for proportions, and to perform Chi-square (or Fisher's exact test) analyses to compare the prevalence of culture-positive *Salmonella* across sampling days within tonsils and each LN type. Differences were deemed significant at a 5% level of significance.

RESULTS AND DISCUSSION

The objective of this project was to estimate the prevalence, in swine presented for slaughter in two municipal (non-federally inspected) abattoirs in Mexico, of culture-positive *Salmonella* in lymphoid tissues: tonsils, and three different lymph node (LNs) types *(Table 1)*. The presence of *Salmonella* in tonsils was 18.0 (9/50) and 40.0% (4/10) in the Merida and Cancun plants, respectively. For mandibular LNs, the overall prevalence was 12.7 (14/110) and 20.0% (2/10) at the Merida and Cancun establishments, respectively. Higher values have been reported by Castagna, Schwarz, Canal, & Cardoso *(4)* in Brazil: 90% (27/30) for tonsils and 36.7% (33/90) for composite tonsil and submandibular LN samples. The occurrence of *Salmonella* in tonsils, mandibular and/or sub-mandibular LNs may reflect contamination during transportation from the farm to the abattoir if situations of animal stress arise that may cause swine to shed *Salmonella* in their feces and contaminate other animals *(4, 14)*. The direct food safety concern with the presence of *Salmonella* in these types of LNs is that they are commonly incorporated into raw pork products, particularly Mexican-style breakfast chorizo, posing a potential risk of disease in humans if the product is not adequately cooked or if cross-contamination occurs at the household or in food service.

On the other hand, the occurrence of *Salmonella* in mesenteric LNs may be an indication of asymptomatic carriage from previous temporal exposure and/or frequent short-term infections, especially at the farm level *(4, 12, 14)*. Our results indicate that in the Merida and Cancun plants, *Salmonella* was present in 44.4 (40/90) and 20.0% (2/10) of mesenteric LNs, respectively. Other authors have reported various prevalence rates for this type of LN: 61% (55/90) in Brazil *(4)*; 21% (85/405) in the southeastern U.S. *(8)*; and 67% (186/278) in Brazil *(12)*. Lastly, our results indicate that 10.2% (12/115) of subiliac LNs collected at the Merida establishment were culture-positive for *Salmonella*. This value is not in accordance with results reported by Wang, Wesley, McKean, & O'Connor *(18)*, who estimated that only 1 of 1,739 (0.06%) subiliac LNs collected in the Midwestern U.S. was *Salmonella*-positive. Contrary to the conclusions of Wang, Wesley, McKean, & O'Connor *(18)*, our results indicate that subiliac LNs may be a suitable candidate for assessment of the safety status of swine and pork products originating at municipal abattoirs in Mexico.

The prevalence of *Salmonella* in LNs different from the ones evaluated in this study has also been reported. Kich et al. *(12)* found that 11% (11/100) of prescapular LNs, another type of deep lymphoid tissue, harbored *Salmonella* in swine slaughtered in Brazil. However, no *Salmonella*-positive prescapular LNs were found by Bahnson, Snyder, & Omran *(1)* in 375 samples collected at a large Midwestern U.S. plant. On the other hand, Gomes-Neves et al. *(9)* studied cross-contamination events in swine abattoirs in Portugal, and estimated that 26.3% (26/99) of ileocecal LNs contained *Salmonella*. All of these results, together with those of the present investigation, suggest that different types of swine lymphoid tissues can harbor viable *Salmonella* bacteria, which in turn may have direct, negative implications for food safety and public health.

Few studies have been conducted to assess the contamination of pork and pork products with *Salmonella* in Mexico. The prevalence in raw pork cuts at retail has been reported: 31.9% (15/47) in Guerrero State over an 11-month period *(2)*; 91.8% (56/61) in Guadalajara City over a one-year period *(6)*; 76% (38/50) in Mexico City over a three-month period *(13)*; 58.1% (197/339) in the Yucatán peninsula area over a two-year period *(21)*; 36.4% in a multi-state study, including Sonora, San Luis Potosí, Michoacán, and Yucatán states *(20)*; and 17.3% (14/81) in Hidalgo State over a

Table 1. Overall prevalence of culture-positive *Salmonella* in different types of lymphoid tissues collected from swine at slaughter in two municipal facilities in Mexico

Type of lymphoid tissue	Facility location			
	Merida		Cancun	
	Prevalence (positive/tested)	95% Confidence Interval (%)	Prevalence (positive/tested)	95% Confidence Interval (%)
Tonsils	18.0 (9/50)	7.4–28.7	40.0 (4/10)	9.6–70.4
Mandibular LNs	12.7 (14/110)	6.5–18.9	20.0 (2/10)	0.0–44.8
Mesenteric LNs	44.4 (40/90)	34.1–54.7	20.0 (2/10)	0.0–44.8
Subiliac LNs	10.2 (12/115)	4.7–15.7	NC	-

NC, not collected

20-month period, including minced pork meat *(15)*. Other raw pork products, particularly chorizo, have been studied to a lesser extent: 72% (18/25) and 20% (5/25) of chorizo prepared on-site at butcher shops and chorizo made industrially in Mexico City, respectively, were *Salmonella* positive *(13)*, whereas 88% (53/60) of chorizo samples collected in Guadalajara City, and 78% (31/40) and 5% (2/40) of chorizo samples collected at butcher shops and grocery stores, respectively, in the city of Querétaro were positive for *Salmonella (5)*. Lastly, only two studies have reported the prevalence of *Salmonella* in raw ground pork: 57.1% (4/7) in Guerrero State *(2)* and 13.9% in three major cities in Mexico – Mexico City, Guadalajara and Monterrey *(16)*. These values indicate that the Mexican population may be at high risk of exposure to *Salmonella* via consumption of contaminated pork products. The contribution of swine tonsils and LNs to the overall prevalence of *Salmonella* in pork products is uncertain; however, it seems possible that given the high burden of *Salmonella* in lymphoid tissues as presented in this study, they may constitute an important source of contamination.

The differences in the results observed in this study from results in the published literature may be attributed to multiple factors, including the existing *Salmonella* status on the farm and surrounding environments; the conditions during transport to the abattoirs, such as duration, sanitation, and stress levels; the conditions at the abattoir, including lairage design, management, and holding time as well sanitary design of the facilities; and regional factors, such as the prevalence of *Salmonella* among herds supplying a specific abattoir *(8, 17)*. The latter may be an explanatory factor for the differences observed in *Salmonella* prevalence across sampling days in the Merida plant *(Fig. 1)*, where values varied significantly across sampling days for all three LN types. In mandibular LNs, values ranged from 0 to 30.0%, in mesenteric LNs from 16.6 to 76.7%, and in subiliac LNs from 0 to 30% *(Fig. 1)*. The finishing step of pigs may be responsible for enhancing *Salmonella* transmission and delivery of positive pig batches with a high number of carriers to the abattoirs. Cross-contamination during transport and lairage remains a concern under contemporary industry conditions, in which efforts to reduce *Salmonella* infection at the farm level may be negated by subsequent infection during transport and lairage *(8)*. Lastly, the partial or complete lack of sanitary and measures observed at both abattoirs may contribute to the results obtained in this study.

CONCLUSIONS

Although this study was conducted with a relatively small set of samples, the results verify that swine carry *Salmonella* systemically in various lymph nodes. This may in turn

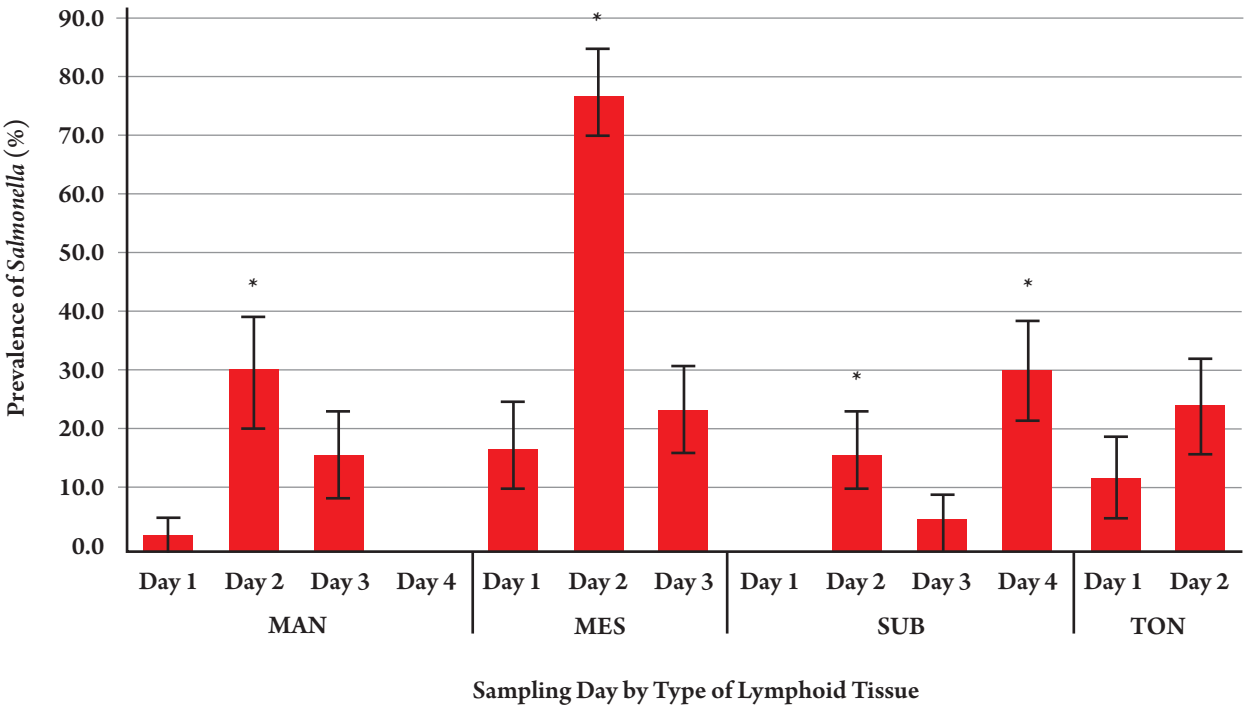

Figure. 1. Day-to-day prevalence of culture-positive Salmonella *in different types of lymphoid tissue collected from swine at slaughter in a municipal facility in Merida, Mexico. MAN = mandibular, MES = mesenteric, SUB = subiliac, TON = tonsils. Bars represent standard error of the proportions with n = 30, 30, 25, 25, 30, 30, 30, 30, 30, 25, 30, 25, and 25, respectively, from left to right. Within tonsils or LN type, asterisks denote a higher proportion at a 5% level of significance.*

represent a risk of contamination of pork products if LNs or tonsils are incorporated into ground beef, sausage-type products such as Mexican style breakfast chorizo, and other products that may be consumed undercooked. To improve food safety and to protect public health, the burden of *Salmonella* in swine lymphoid tissues needs to be reduced. A comprehensive animal welfare, health, and sanitation strategy should be developed and implemented on farms, during finishing of pigs, and at the harvest facility so that a safer and higher quality product may be offered to domestic Mexican consumers.

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