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Research Note

Salmonella enterica in Superficial Cervical (Prescapular) and lleocecal Lymph Nodes of Slaughtered Pigs

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ABSTRACT

Because certain lymph nodes may be incorporated in food products, the presence of *Salmonella enterica* in these tissues could pose a food safety risk. We designed this two-part study to assess the prevalence of *Salmonella* in prescapular lymph nodes from normal slaughtered swine. Prescapular lymph nodes were collected from 300 systematically selected pigs in study 1 and, in study 2, from 75 pigs distributed among 10 herds. For study 2, pooled bacterial cultures were also completed on ileocecal lymph nodes, combining tissue from five pigs per pool (n = 60 pools). No *Salmonella* was detected in study 1 among prescapular lymph nodes (95% confidence interval, 0.0 to 1.16%). *Salmonella* was not detected in 75 prescapular lymph nodes from study 2, although *Salmonella* was detected in 5 of 10 herds in ileocecal lymph nodes. We conclude that prescapular lymph nodes posed a limited food safety risk in this population of pigs.

Salmonella, a foodborne pathogen regulated by the U.S. government (1), has commonly been isolated from the enteric lymph nodes and intestinal contents of slaughterweight pigs (3, 8, 10). However, Salmonella has also been isolated from other lymph nodes, including peripheral lymph nodes in experimental infection (15), and in slaughtered pigs (6, 12, 13). Certain lymph nodes can be incorporated into food products, including pork shoulder and ground products derived from the tissues. Limited data exist on the occurrence of Salmonella in extra-abdominal lymph nodes. In slaughtered sows, Salmonella has been detected in ventral thoracic and subiliac lymph nodes (4 [2%] of 181 samples) (11). At slaughter, Lazaro et al. (12) detected Salmonella in 7 of 98 prescapular lymph nodes (formally, the "superficial cervical center," a collection of lymph nodes lying just cranial to the scapula (14); for consistency with common usage in published literature, we will refer to these nodes collectively as prescapular lymph nodes). Additionally, Lazaro et al. (12) detected Salmonella in 9 of 98 inguinal lymph nodes at three Brazilian slaughter facilities. Narucka (13) reported 5 of 100 carcasses positive in prescapular nodes.

Because Salmonella could pose a threat to food safety if it is found in prescapular lymph nodes and data on Salmonella prevalence at this site were limited, we designed a two-part study. In study 1, our objective was to estimate the prevalence of Salmonella in this lymph node. To ensure that we also sampled from herds with known Salmonella status, in study 2, samples paired at the herd level were

tested for *Salmonella* in both prescapular and ileocecal lymph nodes.

MATERIALS AND METHODS

At one large-scale midwestern U.S. slaughter plant, prescapular lymph nodes were collected from uncured pork shoulders in a two-part study. In study 1, samples were collected systematically from 300 fresh, uncured pork shoulders for approximately 1 month. Samples were collected on Mondays, Tuesdays, and Fridays. Shoulders were selected by use of a 15-min timer; when the timer sounded, one prespecified abattoir worker selected the next ham available on the processing line, placing the ham in a U.S. Department of Agriculture Food Safety Inspection Service-approved sanitized container. Samples were stored at 2°C until the end of the work shift. At this time, lymph nodes were exposed with a knife that had been sanitized in 60°C water, extracted with sterile gauze, placed in individual Whirl-Pak containers, and transported on ice to the laboratory. If lymph nodes were already exposed when shoulders were collected, the sample was not collected because of the risk of cross-contamination.

Samples were pooled, combining 0.5 g of lymph node from each of five individual pigs. Samples were crushed with a mallet, combined with 22.5 ml of lactose broth, and incubated for 24 h at 35°C. A 1-ml sample was transferred to each of tetrathionate (tetrathionate broth base, Remel, Inc., Lenexa, Kans.) and Rappaport-Vassiliadis R10 broth (Remel) and then incubated for 24 h at 35°C. A commercially available enzyme-linked immunosorbent assay kit (TECRA VI, International Bioproducts, Bothell, Wash.) was used to detect a *Salmonella* antigen specific to the genus level.

In study 2, a convenience sample of 10 herds was identified in advance on the basis of participation in a separate study (2) and delivery of at least 30 pigs on days when personnel were available to collect samples. From each of these herds, ileocecal

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lymph nodes were collected from 30 pigs after evisceration. To prevent cross-contamination, the overlying mesentery was reflected before collection with sterile gauze. Two-gram samples of this tissue from each of five pigs were combined and processed as a pooled sample. The first 10 pigs on the processing line were tagged for collection of picnic hams. Prescapular lymph nodes were recovered from 75 pigs by the techniques described. The 25 missed samples were (i) lost to follow-up on the processing line, (ii) unusable because lymph node tissues could not be identified, or (iii) unusable because the lymph node tissue was exposed and therefore potentially contaminated.

All samples in study 2 were processed by modifying a twostep conventional enrichment process and then plated onto selective media (5). For prescapular lymph nodes, 2 g of lymph node tissue was macerated with a mallet and a paddle blender and blended with 20 ml of tetrathionate broth (Remel). For ileocecal lymph nodes, 2 g from each of five pigs were combined, macerated, and blended with 90 ml of tetrathionate broth. The tetrathionate broths were incubated for 42 to 48 h. One milliliter of broth was transferred to 9 ml of R10 broth (Remel) and incubated for 24 h. Broths were streaked for isolation on XLT4 agar (XLT4 agar base, Remel). If a suspect colony was found after 24 h of growth, one colony per sample was streaked to brilliant green agar and then incubated for 24 h. All incubations were at 37°C. Suspect colonies were tested for agglutination with anti-Salmonella antibodies (polyvalent O groups A through G Salmonella somatic agglutinating serum, rabbit, Remel). Isolates that did not agglutinate were further tested with a commercial test kit (API 20E, bioMérieux, Inc., Hazelwood, Mo.). Isolates that were found positive either by agglutination or the test kit were serotyped at either the U.S. Department of Agriculture National Veterinary Services Laboratory (Ames, Iowa) or the Wisconsin Veterinary Diagnostic Laboratory (Madison).

We used StatXact 4.0 (Cytel Software, Inc., Cambridge, Mass.) to estimate exact confidence intervals for prevalence in prescapular lymph nodes and used the procedure described by Casella (4).

RESULTS

No Salmonella was detected among any of the 300 samples processed in study 1 (95% confidence interval, 0.0 to 1.16%). Among the 10 herds in study 2, Salmonella was detected in five herds from pooled ileocecal lymph node samples (15 of 60 pooled samples). The serovars and number of isolates detected were as follows: Salmonella Derby, five; Salmonella Typhimurium (Copenhagen), four; Salmonella Java, two; Salmonella Hartford, one; Salmonella Mbandaka, one; and Salmonella Senftenberg, one. One additional isolate was not serotyped. Salmonella was not detected in any of the 75 prescapular lymph nodes from a subset of the same pigs.

DISCUSSION

In study 2, a minimum of 15 of the 300 individual ileocececal lymph nodes were *Salmonella* positive, given that *Salmonella* was detected in 15 pooled samples. This contrasts with a failure to detect *Salmonella* in any of the 75 prescapular nodes sampled from a subset of the same pigs. None of the randomly selected prescapular nodes were culture positive in study 1, which further suggests that prescapular nodes were a minor source of *Salmonella* in these individuals. Differing laboratory methods were used in

studies 1 and 2. However, the detection kit used in study 1 has been reported to be at least as sensitive as a conventional culture protocol for diagnostic isolates (9).

Although Salmonella has been found in lymph nodes distant from the gut, Wood et al. (15) reported finding Salmonella in such lymph nodes no longer than 2 weeks postinfection. A study of 560 slaughtered pigs in Bulgaria (7) reported no Salmonella in prescapular lymph nodes. In contrast, two studies have reported culture-positive prescapular lymph nodes (12, 13). However, neither positive report described using sanitary or sterile techniques when the samples were collected, but instead, they reported steps to decontaminate the surface of the nodes, holding open the possibility that cross-contamination may have occurred during sample collection. In addition, Salmonella was detected in 30 of 100 mesenteric lymph nodes reported by Narucka (13), which is higher than the prevalence in ileocecal nodes reported in the current study. Because ileocecal lymph nodes are a subset of the mesenteric lymph node chain, this suggests either higher overall infection or contamination rates in the Narucka (13) study. The slaughter plants reported by Lazaro et al. (12) were small in capacity, and one was described as having "dubious" hygienic conditions, with variable and, in some cases, extended lairage times

Prescapular nodes represented a low risk even among individuals with culture-positive gut-associated lymph nodes. This may be expected, because ileocecal nodes drain the cecum, a section of gut relatively favorable to *Salmonella*. Although the potential for *Salmonella* to be found in prescapular lymph nodes remains, the estimated prevalence of 0% (95% upper bound, 1.2%) in the current study suggests that these lymph nodes represented a minor risk to food safety among this study population.

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