Survey of Intact and Nonintact Raw Pork Collected at Retail Stores in the Mid-Atlantic Region of the United States for the Seven Regulated Serogroups of Shiga Toxin–Producing *Escherichia coli*

YANGJIN JUNG, ANNA C. S. PORTO-FETT, BRADLEY A. SHOYER, LAURA E. SHANE, ELIZABETH HENRY, MANUELA OSORIA, AND JOHN B. LUCHANSKY*

U.S. Department of Agriculture, Agricultural Research Service, 600 East Mermaid Lane, Wyndmoor, Pennsylvania 19038, USA

MS 19-192: Received 22 April 2019/Accepted 30 June 2019/Published Online 10 October 2019

ABSTRACT

A total of 514 raw pork samples (395 ground or nonintact and 119 intact samples) were purchased at retail stores in Pennsylvania, Delaware, and New Jersey between July and December 2017. All raw pork samples were screened for serogroup O26, O45, O103, O111, O121, O145, or O157:H7 cells of Shiga toxin–producing *Escherichia coli* (STEC-7) using standard microbiological and molecular methods. In short, 21 (5.3%) of the 395 ground or nonintact pork samples and 3 (3.4%) of the 119 intact pork samples tested positive via the BAX system real-time PCR assay for the *stx* and *eae* virulence genes and for the somatic *O* antigens for at least one of the STEC-7 serogroups. However, none of these 24 presumptive-positive pork samples subsequently yielded a viable isolate of STEC displaying a STEC-7 serogroup–specific surface antigen in combination with the *stx* and *eae* genes. These data suggest that cells of STEC serogroups O26, O45, O103, O111, O121, O145, or O157:H7 are not common in retail raw pork samples in the mid-Atlantic region of the United States.

HIGHLIGHTS

- None of the 514 retail raw pork samples were positive for STEC-7.
- Four of 514 raw pork samples harbored *E. coli* of unknown serogroup containing *stx* and *eae*.
- STEC-7 are uncommon in retail raw pork samples in the U.S. mid-Atlantic region.

Key words: Ground pork; Nonintact pork; Pork cuts; Shiga toxin–producing *Escherichia coli*

According to the U.S. Department of Agriculture (USDA), Food Safety and Inspection Service (FSIS) (46), pork is the most consumed meat worldwide. Pork remains a safe and wholesome food due, in part, to longstanding and consistent messaging by public health agencies to educate consumers about adequately cooking pork to lessen the likelihood of contracting porcine-specific foodborne illnesses, notably trichinosis. Parasites such as *Trichinella spiralis*, the etiologic agent of trichinosis, and more recently, *Toxoplasma gondii* are most often perceived by consumers as the disease agents of greatest concern for foodborne illness related to pork (2, 3, 15). In reality, bacterial pathogens such as *Salmonella* and *Campylobacter*, as well as *Yersinia enterocolitica*, *Staphylococcus aureus*, and *Listeria monocytogenes*, cause a far greater number of illnesses and deaths, as well as costly product recalls, attributed to pork each year in the United States than the abovementioned parasites (37, 46). Among bacterial pathogens that are associated with swine and that subsequently infect humans due primarily to insufficient cooking, improper handling, or cross-contamination of pork products, in recent years Shiga toxin–producing cells of *Escherichia coli* (STEC) have been more commonly observed on and in swine and have been more frequently associated with human illness (41). Although pork is not a common vehicle for foodborne illness due to STEC (39, 41), over the last 25 years there have been a handful of STEC outbreaks caused by pork products, including meat from barbecued pork, a roasting pig, salami, and mettwurst; the latter two product types are a mixture of pork with beef and/or lamb (10, 13, 20, 26, 27, 40, 50). Therefore, both researchers and regulators have stated that further research is warranted to determine whether the presence of STEC and on swine in general, and in and on pork products specifically, is a significant, and likely expanding, public health concern (17, 41).

Uncooked and improperly handled beef products have been a common vehicle for illnesses caused by STEC for almost 40 years, and numerous investigators have reported recovery of this pathogen from intact and nonintact beef subprimals (recovery rates of ca. <0.083 to 0.2% at levels of <0.375 CFU/cm²) and ground beef (recovery rates of 0.1 to 54.2% at levels of 0.5 to 4.0 log/CFU) (1, 9, 21, 33, 35).
Although STEC isolates are also recovered from numerous other livestock and associated food products, including swine and pork products (29), at present, only cells of the seven serogroups of STEC (STEC-7; O26, O45, O103, O111, O121, O145, and O157:H7) are considered adulterants when they are present in raw ground or nonintact beef, but not in raw pork (45). That being said, the incidence of STEC on swine and swine carcasses at slaughter has been estimated at 0.4 and 4.8%, respectively (5, 6, 12, 24). Cells of STEC have also been recovered from raw pork products at slaughter and processing facilities at a frequency of 0.2 to 5.0% (12, 19, 38), as well as from raw pork at food retailers (1.5 to 18% (39)). In many of the studies published to date, isolates recovered from pigs or pork harbor the requisite virulence genes for STEC (i.e., stx, eae, and ehx) or display the somatic O and flagellar H antigens for the regulated serogroups of STEC (i.e., O26, O45, O103, O111, O121, O145, or O157:H7); however, compared with beef, relatively few isolates recovered from pork products have encoded for both the essential virulence genes and signature surface antigens for STEC-7. For many reasons, therefore, the true prevalence of the seven serogroups of STEC regulated for beef, and that may also be associated with pork products, remains unclear.

The primary objective of the present study was to quantify the recovery rate of the seven regulated serogroups of STEC from raw pork at retail. A secondary objective was to quantify the levels and types of STEC found within pork samples testing positive for the pathogen. A final objective was to assess whether porcine isolates of STEC recovered from the raw pork samples analyzed herein were linked to human illness.

MATERIALS AND METHODS

Collection of raw pork samples and associated metadata. Members of our research team visited retail stores in Pennsylvania, New Jersey, and Delaware between July and December 2017 and purchased raw pork products. Shoppers were instructed to collect metadata and to purchase ground pork and pork cuts (ca. 0.5 to 1.5 kg per sample; intact and nonintact cuts of pork) within the use by or sell by date, and to place each sample in a separate slide seal zip bag (S2pzX10, G. T. Company, Petaluma, CA). The temperature of each pork sample was recorded at the time of purchase, using a handheld infrared thermometer (Traceable, Fisher Scientific, Pittsburgh, PA). Bagged products were placed into coolers with ice packs and transported by car (≤2 h) to the USDA, Agricultural Research Service, Eastern Regional Research Center (USDA ARS ERRC, Wyndmoor, PA). Upon arrival at the laboratory, samples were stored at 4°C for up to 3 days until processed. Each sample was repacked and coded by laboratory personnel who were not shoppers and who were not involved in the microbiological analyses of the raw pork. Metadata collected by the shoppers included sampling date, store name and location, product information on a package, and temperature of the products upon purchase.

Screening of raw pork for STEC-7 and recovery of isolates from presumptive-positive samples. As depicted in Figure 1, pork was analyzed for STEC using the FSIS Microbiology Laboratory Guidebook (method 5C.00 (48)) with only minor modifications. In brief, a 325 ± 5 g sample of meat was enriched in 975 ± 5 mL of modified tryptic soy broth (mTSB; Oxoid, Hants, UK) with added casamino acids (10 g/1 L of mTSB; VWR, Solon, OH) at 42°C for 18 to 22 h. Next, enriched samples were screened for STEC-7 using the BAX real-time PCR assay (BAX System Q7, DuPont Qualicon, Wilmington, DE) according to the manufacturer’s instructions. The initial BAX assay is specific for the stx and eae virulence genes, and subsequent somatic antigen panels for the BAX assay are specific for each of the regulated seven serogroups of STEC.

When samples tested positive for stx, eae, and any of the seven regulated serogroup antigen genes via BAX, portions of the original enrichment broth were then subjected to immunomagnetic separation. Briefly, a 20-μL aliquot of serogroup-specific antigen beads for STEC-7 (Abraxis, Warminster, PA) was individually added to a 980-μL aliquot of the presumptive-positive enrichment broth. Samples were then subjected to immunomagnetic separation using a Kingfisher Flex Magnetic Particle Processor (type 711, Thermo Scientific, Waltham, MA) according to the manufacturer’s instructions. Following immunomagnetic separation, a 50-μL aliquot of the eluted STEC-7 bead sample, with or without a 10-fold dilution in sterile distilled water, was spread plated onto modified Possé agar plates (31) with the aid of a plastic L-shaped spreader (model SPR-L-S10, Excel Scientific, Victorville, CA). Numerous representative STEC-7 colonies (based on color and morphology) from the Possé plates were randomly picked and suspended separately in 100 μL of sterile water. A 50-μL aliquot of each isolate suspension was tested individually by an 11-multiplex PCR (4) to identify and recover STEC-7 isolates displaying any of the seven serogroup genes and harboring any of the four virulence genes (stx1, stx2, eae, and ehxA). Any isolates that possessed surface antigens for any of the seven regulated serogroups and that encoded for stx1 and/or stx2 and eae were considered to be STEC. Any isolates harboring at least one of the 11 target genes (i.e., genes for seven somatic O antigens or stx1, stx2, eae, or ehxA) during the confirmation step were retained at −80°C in brain heart infusion broth (Difco, BD, Franklin Lakes, NJ) plus 10% glycerol (Fisher Bioreagents, Fair Lawn, NJ) essentially as described (25). For quality control purposes, five environmental samples, randomly selected from different food contact surfaces, nonfood contact surfaces, or personnel equipment in our laboratory (20 sites total to select or sample from), were obtained via sponge, processed as described previously (25), and tested for STEC-7 biweekly during the sampling process as described above.

RESULTS AND DISCUSSION

Pork is a known, but not particularly common, source of foodborne illness due to cells of serotype O157:H7 STEC (10, 13, 20, 27, 40, 50). With the exception of an outbreak in Nebraska linked to barbecued pork caused by a serogroup O111 isolate of STEC, there have been no documented illnesses attributed to pork caused by the seven regulated serogroups of STEC (O26, O45, O103, O111, O121, O145, and O157:H7). Although STEC pathogens are considered adulterants in raw ground or nonintact beef, but not in pork, E. coli strains producing Shiga toxins have been recovered from swine and pork products at both swine slaughter and pork processing plants (5, 11, 12, 19, 24). However, such isolates have only rarely displayed both the target somatic antigens and the associated virulence genes to be confirmed as one of the seven serogroups of STEC regulated for beef that may also be found in swine and pork.
In the present study, 514 pork samples (395 ground or nonintact and 119 intact samples), representing 60 brands, were purchased at 107 retail stores in the mid-Atlantic region of the United States. These food retailers consisted of large chain stores, independent grocery stores, butcher shops, and farmers’ markets that were visited between one and six times each, for a total of 232 store visits, during 16 shopping trips between July and December 2017. The average temperature of the 514 pork samples at the time of purchase was 4.9 ± 2.5°C (range, −6.1 to 12.8°C). The majority of product labels (490 of 514, 95.3%) displayed the required “safe handling instructions” for raw meats on the label, and ca. 228 (44.4%) of the 514 sample labels displayed “cooking instructions” with specific times and temperatures and/or recommendations for using a food thermometer to measure the internal temperature. Note that the 24 samples (21 ground and 3 cuts) that did not display “safe handling instructions” and/or “cooking instructions” on the label were purchased at farmers’ markets or butcher shops. The average temperature of these 24 samples at the time of purchase was 7.2 ± 2.9°C (range, 0 to 11.7°C), which on average, is noticeably higher than for the overall average temperature of the 514 samples taken at point of purchase.

None of the 514 pork samples (395 ground or nonintact and 119 intact samples) purchased at retail were subsequently confirmed as positive for any of the seven regulated serogroups of STEC. More specifically, of the 514 samples, 47 (9.1%) tested positive for stx and 136 (26.5%) tested positive for eae by the BAX assay, whereas 33 (6.4%) tested positive for both of these virulence genes. Of the 33 samples testing positive for stx and eae, 24 tested positive via BAX for at least one, and up to four, of the STEC-7 serogroups (Table 1). The most prevalent serogroups from these samples were serogroups O145 (12 samples) and O121 (12 samples), followed by serogroups O45 (9 samples), O103 (6 samples), and O26 (5 samples), and then serogroup O111 (1 sample) and serotype O157:H7 (1 sample) (Table 2). Next, these 24 samples, which tested positive via the BAX assay for stx and eae and also displayed surface antigens for at least one of the STEC-7 serogroups, were further screened for a viable isolate of STEC-7 by subjecting a portion of the original enrichment broth to immunomagnetic separation and plating portions of the resulting eluate onto Possé agar plates (Fig. 1). Multiple isolates (847 total isolates from 24 samples) from each sample that displayed the colony size, shape, and color typical for STEC-7 were retained for further characterization. Of these 847 isolates screened by an 11-plex PCR, none harbored any of the target somatic antigens for the regulated STEC-7 serogroups in combination with the stx and eae genes needed for virulence (Table 2). Nonetheless, 66 of 847 isolates were retained from 13 of the 24 samples because such isolates tested positive for at least one of the following 11 genes: stx₁, stx₂, eae, ehxA, or one of the somatic antigens of the seven serogroups (Table 3).
specifically, the somatic antigen for 34 (51.5%) of the 66 isolates remains undetermined, whereas 32 (48.5%) of the 66 isolates tested positive for the somatic antigen from serogroups O145 (12 isolates), O26 (8 isolates), O45 (6 isolates), O103 (5 isolates), or O121 (1 isolate). Of the 34 isolates for which the somatic antigen was undetermined, 17 (50%) harbored the stx and eae genes, 1 (2.9%) contained only stx, 2 (5.9%) contained stx and ehxA, and 7 (20.6%) contained the eae and ehxA genes. Of the 32 isolates that harbored the somatic antigen for serogroups O145, O26, O45, O103, and O121, 11 (34.4%) did not harbor any of the four virulence genes (stx, stx2, eae, or ehxA), 16 (50%) harbored eae and ehxA, and 5 (15.6%) harbored only ehxA.

Although cells of serotype O157:H7 STEC and cells of the other six regulated non-O157 serogroups were not recovered from nonintact or intact pork samples collected from retail stores in the mid-Atlantic region of the United States, 17 isolates recovered from 4 (0.78%) of 514 raw pork samples were shown by the 11-plex PCR assay to harbor the stx and eae genes and, thus, were capable of causing human illnesses (Table 3). Two of these four samples were from the same brand, but were purchased at

---

**TABLE 2.** Screening for STEC-7 isolates recovered from enrichment of 24 presumptive-positive pork samples

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Meat type</th>
<th>stx</th>
<th>eae</th>
<th>Serogroup(s)</th>
<th>Screened</th>
<th>Retained</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA67-5</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O121, O45</td>
<td>31</td>
<td>None</td>
</tr>
<tr>
<td>PB56-6</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O145</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>PDD82-6</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O45, O145</td>
<td>19</td>
<td>5</td>
</tr>
<tr>
<td>PF4</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O26, O145</td>
<td>46</td>
<td>7</td>
</tr>
<tr>
<td>PG68</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O121, O45, O103</td>
<td>58</td>
<td>5</td>
</tr>
<tr>
<td>PG75</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O121, O145</td>
<td>36</td>
<td>7</td>
</tr>
<tr>
<td>PJ9</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O121</td>
<td>19</td>
<td>None</td>
</tr>
<tr>
<td>PQQ53</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O121</td>
<td>39</td>
<td>7</td>
</tr>
<tr>
<td>PQQ53-2</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O145</td>
<td>48</td>
<td>None</td>
</tr>
<tr>
<td>PQQ53-3</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O121, O103, O145</td>
<td>46</td>
<td>1</td>
</tr>
<tr>
<td>PQQ66-2</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O45</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>PQQ66-3</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O103, O145</td>
<td>28</td>
<td>5</td>
</tr>
<tr>
<td>PQQ69</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O145</td>
<td>27</td>
<td>None</td>
</tr>
<tr>
<td>PQ7</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O45</td>
<td>19</td>
<td>None</td>
</tr>
<tr>
<td>PQQ90</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O45</td>
<td>23</td>
<td>5</td>
</tr>
<tr>
<td>PV21-16</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O26, O103</td>
<td>25</td>
<td>None</td>
</tr>
<tr>
<td>PV21-6</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O26, O45, O103</td>
<td>49</td>
<td>4</td>
</tr>
<tr>
<td>PV60-5</td>
<td>Ground</td>
<td>+</td>
<td>+</td>
<td>O121</td>
<td>31</td>
<td>None</td>
</tr>
<tr>
<td>PW25</td>
<td>Nonintact</td>
<td>+</td>
<td>+</td>
<td>O121, O45, O145</td>
<td>64</td>
<td>None</td>
</tr>
<tr>
<td>PC56-3</td>
<td>Nonintact</td>
<td>+</td>
<td>+</td>
<td>O121</td>
<td>22</td>
<td>None</td>
</tr>
<tr>
<td>PS16</td>
<td>Nonintact</td>
<td>+</td>
<td>+</td>
<td>O26, O121, O145</td>
<td>59</td>
<td>None</td>
</tr>
<tr>
<td>PR11</td>
<td>Intact</td>
<td>+</td>
<td>+</td>
<td>O111, O121, O103, O145</td>
<td>42</td>
<td>5</td>
</tr>
<tr>
<td>PP13-5</td>
<td>Intact</td>
<td>+</td>
<td>+</td>
<td>O26, O121, O45</td>
<td>35</td>
<td>8</td>
</tr>
<tr>
<td>PUU10-2</td>
<td>Intact</td>
<td>+</td>
<td>+</td>
<td>O145, O157</td>
<td>43</td>
<td>None</td>
</tr>
</tbody>
</table>

Total 24

| No. of isolates | 847 | 66 |

---

a Samples shown to harbor both stx and eae genes along with the target serogroup-specific O antigen as determined using the BAX real-time PCR assay (Table 1).

b Isolates recovered from 24 presumptive-positive samples by the BAX assay that were screened for stx and eae along with the target serogroup-specific O antigen by the 11-plex PCR (4).

c Isolates retained from 13 of the 24 presumptive-positive samples that tested positive for at least one of the following 11 genes: stx1, stx2, eae, ehxA, or each of the somatic antigens of the seven serogroups.
different times from different locations of a large chain store (New Jersey, one sample; Delaware, one sample). Both samples displayed the “safe handling instructions” label, but not the “cooking instructions” label. The other two samples were purchased at different times, from two different stores (one large chain and one independent store) located in New Jersey; only one sample displayed the “safe handling instructions” along with the “cooking instructions” label. Although these 17 isolates harbored genes for 
\textit{stx} and \textit{eae}, none of these isolates displayed the serogroup-specific O-antigens for STEC-7. For these reasons, efforts should be made to determine the specific serotype (and clonality) of these 17 isolates from the three ground (16 isolates) and one intact (1 isolate) raw pork samples to ascertain whether these serotypes have caused human illnesses from pork or other foods.

Other investigators isolated STEC from beef, pork, and lamb products from food retailers at recovery rates that ranged from 3.7 to 40.8\%, 1.5 to 18.0\%, and 17.1 to 48.0\%, respectively (7, 14, 32, 35, 36). For example, Samadpour et al. (36) reported that of 51 retail pork samples screened for Shiga-like toxin-producing \textit{E. coli}, 2 isolates were positive for Shiga-like toxin (SLT I, 6 isolates were positive for SLT II, and 1 isolate was positive for both SLT I and SLT II. In another study, Xia et al. (51) screened 1,167 \textit{E. coli} isolates recovered from pork chops purchased at retail markets in the United States between 2002 and 2007; only 1 isolate (0.09\%), serotyped as ONT:H51, was positive for \textit{stx}1. In a similar study, Magwedere et al. (28) reported that 8 of 16 ground pork samples collected at retail in the United States tested positive for the presence of the target somatic antigens for STEC-7 (O121 [6 samples], O157 [1 sample], and O103 [1 sample]); none of these 8 samples tested positive for \textit{stx} genes. Likewise, Ju and colleagues (23) screened 231 ground pork samples purchased at chain grocery stores for non-O157 STEC; none of these samples tested positive for any of the six somatic antigens of the regulated non-O157 STEC. However, of the 231 samples, 31 (13.4\%) tested positive for the \textit{stx} genes via PCR and 12 (5.2\%) tested positive for \textit{stx} genes via colony hybridization. According to the authors, of the 16 isolates that were recovered from the samples positive for \textit{stx}, 8 isolates displayed serogroup O91, whereas the remaining 8 isolates were of unknown serogroup. In Italy, Ercoli et al. (16) found that, among 675 fresh and dried pork samples collected at processing plants, 19 samples (fresh pork sausage) tested positive for the \textit{stx}1 or \textit{stx}2 genes; of these, 10 tested positive for the \textit{stx}2 and \textit{eae} genes. These authors reported that the most prevalent serogroup isolated was serogroup O145; however, no isolates were recovered from the 10 presumptive-positive samples that harbored the \textit{stx}1, \textit{stx}2, or \textit{stx}2 genes in combination with the \textit{eae} gene.

Although cells of serotype O157:H7 STEC and cells of the other six regulated serogroups of STEC have been declared “adulterants” in raw, nonintact beef products, STEC-7 are not, to date, considered adulterants in raw, ground or nonintact further processed pork products (42, 43). Given the recovery of STEC from swine and pork products and given the handful of STEC outbreaks attributed to pork, it is essential that pork be cooked to an internal temperature of 145°F (ca. 62.8°C) and held for 3 min (i.e., for roasts, hams, and chops) or be cooked to an instantaneous internal temperature of 160°F (ca. 71.1°C) (i.e., for ground pork) to lower the potential risk of foodborne illness from either parasitic (e.g., trichinae and toxoplasmas) or bacterial pathogens (e.g., \textit{Salmonella}, STEC, and \textit{L. monocytogenes}) (44, 46). To the best of our knowledge, no studies have been published on the comparative prevalence of serotype O157:H7 and the “Big Six” regulated serogroups of STEC associated with raw pork cuts at retail establishments in the United States. However, some investigators have established that STEC are recoverable from swine, that STEC can be shed for a couple of months by infected swine, and that swine can transfer STEC to and from other livestock species (41). Investigators in Japan (30) and Norway (22) established the recovery rate of STEC O157:H7 in pig feces at 1.4 and 0.1\%, respectively, whereas Bush (8) reported that it was not possible to recover STEC O157:H7 from pig feces in the United States. In another study, Feder and colleagues (18) recovered cells of STEC O157:H7 that encoded for \textit{eae}, \textit{stx}1, and \textit{stx}2 or \textit{eae}, \textit{hly}; and \textit{stx}1 from 6 (2.0\%) of 305 intact colon fecal samples from swine harvested at an abattoir in the United States. Rios et al. (34) also recovered STEC by direct sampling of the intestinal content of pigs at an abattoir in Chile. Collectively, as reported by our group and other investigators, swine hides, carcasses, and intestinal contents, as well as raw pork, can serve as

**Table 3. Characterization of the 66 isolates recovered from 13 presumptive-positive samples via an 11-gene mPCR**

<table>
<thead>
<tr>
<th>Sample code</th>
<th>No. of isolates&lt;sup&gt;a&lt;/sup&gt;</th>
<th>\textit{stx}&lt;sub&gt;1&lt;/sub&gt;</th>
<th>\textit{stx}&lt;sub&gt;2&lt;/sub&gt;</th>
<th>\textit{eae}</th>
<th>\textit{ehxA}</th>
<th>Serogroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR11, PQQ90</td>
<td>9</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Unknown&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>PQQ53</td>
<td>7</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Unknown</td>
</tr>
<tr>
<td>PV21-6</td>
<td>1</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>—</td>
<td>Unknown</td>
</tr>
<tr>
<td>PG75</td>
<td>2</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>Unknown</td>
</tr>
<tr>
<td>PG68, PV21-6</td>
<td>8</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>—</td>
<td>Unknown</td>
</tr>
<tr>
<td>PB56-6, PF-4, PQQ66-2, PQQ90, PP13-5</td>
<td>23</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>+</td>
<td>Unknown, O26, O45, O145</td>
</tr>
<tr>
<td>PG75</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>+</td>
<td>—</td>
<td>O145</td>
</tr>
<tr>
<td>PDD82-6, PQQ53-3, PQQ66-3</td>
<td>11</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>O45, O103, O121</td>
</tr>
</tbody>
</table>

<sup>a</sup> Isolates were screened according to Bai et al. (3) using an 11-gene multiplex PCR to detect the seven regulated STEC serogroups and four major virulence factors ( intimin [\textit{eae}], enterohemorrhagic hemolysin [\textit{ehxA}], and Shiga toxins [\textit{stx}1 and \textit{stx}2]) genes.

<sup>b</sup> +, gene detected; —, gene not detected.

<sup>c</sup> Unknown, no STEC-7 specific O-antigen genes determined.
vehicles for transmission of STEC. However, based on the lack of scientific or epidemiological data to the contrary, and in light of our findings, it would seem that the risk to human health caused by STEC in retail pork is relatively low.

Isolates of *E. coli* recovered from the 514 raw pork samples screened herein did not harbor the requisite virulence genes (i.e., *stx* and *eae*) in combination with the specific somatic antigens (i.e., O26, O45, O103, O111, O121, O145, or O157:H7) to be identified as STEC-7. That being said, as shown by an 11-plex PCR, at least 17 isolates encoding for *eae* warranted to determine the serotype of these 17 isolates properly. Further studies are needed to assess whether these isolates may have previously caused human illnesses from pork or other foods. Additional studies are also planned to collect and sample a far greater number of raw intact and nonintact pork samples from food retailers to gain insight on the true prevalence of the regulated serogroups of STEC in pork and to enumerate pathogen levels. Such data should shed appreciable light on whether illnesses due to STEC linked with pork are more likely owing to contamination of pork during swine processing followed by inadequate cooking and handling or whether they are more likely due to cross-contamination just prior to human contact or consumption.

**ACKNOWLEDGMENTS**

We extend our appreciation to Steve Campano (Hawkins Inc., Rosedale, MN) for helpful discussions and technical support. This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award no. 2012-68003-30155. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

**REFERENCES**


