Comparison of Culture, Multiplex, and 5′ Nuclease Polymerase Chain Reaction Assays for the Rapid Detection of Yersinia enterocolitica in Swine and Pork Products†

SANIDHYA BOYPALLE,1,2 IRENE V. WESLEY,1,* H. SCOTT HURD,1 AND P. GOPAL REDDY2

1Pre-Harvest Food Safety and Enteric Diseases Research Unit, National Animal Disease Center, USDA, Agriculture Research Service, Ames, Iowa 50010; and 2Tuskegee University, Tuskegee, Alabama 36088, USA

ABSTRACT

Bacteriological culture was compared with multiplex and fluorogenic (TaqMan) polymerase chain reaction (PCR) assays for the detection of attachment invasion locus (ail)-bearing Yersinia enterocolitica in market weight swine, chitterlings, and ground pork. The TaqMan assay detected 1 pg of purified Y. enterocolitica DNA, whereas conventional gel-based PCR detected 1 ng of the same. The presence of ail-bearing Y. enterocolitica was tested in pork and feces artificially inoculated with Y. enterocolitica strain NADC 5561. The sensitivity limits of culture, multiplex, and TaqMan PCR assays were $4 \times 10^3$, $4 \times 10^2$, and 0.4 CFU/g, respectively, for the artificially inoculated pork. The sensitivity limits were $4 \times 10^2$, $4 \times 10^2$, and 0.4 CFU/g, respectively, for feces after a 48-h enrichment in a Yersinia selective broth. By the culture method, Y. enterocolitica was not detected in any of the swine samples (n = 2,403) examined. By contrast, it was detected in 48 (2%) of the swine samples screened using the multiplex PCR and in 656 (27.2%) of these samples using the TaqMan assay. Using the culture method, Y. enterocolitica was detected in 8% of chitterling samples (n = 350) and in none of the ground pork samples (n = 350). It was identified in 27% of the chitterling samples using multiplex PCR and in 79% of these samples using the TaqMan assay. Ten percent of the ground pork samples contained Y. enterocolitica, as determined by the multiplex PCR, and 38% based on the TaqMan assay. The results suggest that pork products harbor more ail-bearing Y. enterocolitica than selected organs of freshly slaughtered hogs and that the TaqMan assay is more sensitive than either the multiplex PCR or traditional culture methods.

In the United States, Yersinia enterocolitica is estimated to cause 3,000 to 20,000 cases of human disease annually (16). Active Surveillance Network (FoodNet) data for 1999 show the incidence of yersiniosis at 0.8 per 100,000 population in sentinel U.S. sites (35). Children under 5 years old are particularly susceptible, while older children and adults may manifest enterocolitis, severe abdominal pain mimicking appendicitis, mesenteric lymphadenitis, and systemic infections followed by autoimmune complications such as reactive arthritis and erythema nodosum (10). Outbreaks of human yersiniosis caused by Y. enterocolitica have been associated with the consumption of milk contaminated with pig or human feces during or after processing (44), untreated fresh water (39), bean sprouts (10), and pork products (11).

Y. enterocolitica is considered a primary foodborne pathogen in Belgium, Canada, Japan, Denmark, and Finland (11). The predominant serotypes implicated in human illness in Europe are O:3 and O:9 (45), while in United States, O:8 and O:5 predominate (26). Recent outbreaks in the United States have shown O:3 infections in infants attributed to household preparation of chitterlings (an ethnic food prepared from hog intestines) (29), suggesting a serogroup shift (38).

Pathogenic strains have been repeatedly isolated from tonsils, tongues, cheek meat, and intestinal tracts of healthy pigs, as well as from raw pork and pork products (11, 21). In countries where Y. enterocolitica is a major foodborne pathogen, the carriage rates in swine range from approximately 35 to 70% of herds, with an estimated 4.5 to 100% of individual swine carrying human pathogenic strains (18). Funk et al. (21) reported that out of 3,375 pigs from 103 lots at slaughter sampled by oral-pharyngeal swabbing, 29 lots (28.2%) contained at least one pig from which attachment invasion locus (ail)-bearing Y. enterocolitica was isolated.

Yersiniosis attributed to consumption of raw or undercooked pork products was first described by Tauxe et al. (49) in a study conducted in Belgium, which showed that 18% of children eating raw pork were affected with yersiniosis. A case-control study by Ostroff et al. (39) performed in 67 case-patients (mean age, 23.4 years; range, 8 months to 88 years), implicated undercooked pork, sausage products, and untreated water as significant risk factors for yersiniosis.

The pathogenicity of Y. enterocolitica is multifactorial. The virulence plasmid pYV is well conserved among the pathogenic species (40). Plasmid pYV harbors genes for the production of yadA, an outer membrane protein, which mediates binding of the pathogen to the Hep-2 cells in the...
ileocecal region of the gut of the host (32). Other virulence factors of *Y. enterocolitica* are the chromosomally encoded *inv* (invasion) and *ail* (34). The *ail* gene is characteristic of pathogenic *Y. enterocolitica*, and *ail*-bearing isolates have been recognized in human clinical material and also healthy swine (33).

Polymerase chain reaction (PCR) assays have been developed that target the chromosomally encoded genes: *ail* (5, 15, 27, 28); *rfbc* (55); *yst* (23); *inv* (41); 16SrRNA (50); and plasmidborne genes *virF* (15, 28, 56) and *yadA* (5, 25) of *Y. enterocolitica*.

The fluorogenic PCR (TaqMan) assay is a PCR-based protocol that eliminates post-PCR processing for detection of amplified products. Lockey et al. (31) reported that the TaqMan PCR assay can achieve the same sensitivity as the time-consuming hybridization techniques without the need for further analysis of the PCR products. The assay exploits the 5'-3' nuclease activity of Taq polymerase to hydrolyze an internal hybridization probe labeled with a fluorescent reporter dye at the 5' end and a quencher dye at the 3' terminal (22, 30). Hydrolysis during amplification releases the reporter dye from the probe, thus reducing the quenching effect and allowing for an increase in the emission of the reporter fluorescence. This increase in fluorescence is a direct result of amplification of target DNA (22). The assay is more sensitive and specific than conventional PCR in detecting pathogens (8, 14). The assay has been used to detect *Listeria monocytogenes*, *Escherichia coli O157:H7*, *Salmonella* spp., and *Campylobacter jejuni* in a variety of food matrices (3, 6, 8, 37, 46). Recently, we reported the development of the fluorogenic PCR assay amplifying the *ail* locus of *Y. enterocolitica*, which was adapted in the present study (24).

The goal of this study was to compare the sensitivity limits of the culture method and PCR-based assays (multiplex and TaqMan) to determine the most rapid and sensitive method for the detection of *ail*-bearing strains of *Y. enterocolitica* in swine and pork. We also wished to estimate the prevalence of *Y. enterocolitica* in market weight swine, chitterlings, and ground pork by the traditional culture method, as well as by multiplex and TaqMan PCR assays.

**MATERIALS AND METHODS**

**Bacterial strains.** *Y. enterocolitica* strain NADC 5561, serotype O:9, was obtained from the Centers of Disease Control (Atlanta, Ga.). *Y. enterocolitica* strain NADC 5233 (serotype not known) and non-*Y. enterocolitica* strains including *Yersinia aldovae*, NADC 5612; *Yersinia bercovieri*, NADC 5615; *Yersinia frederiksenii*, NADC 5617; and *Yersinia kristensenii*, NADC 5622 were obtained from the American Type Culture Collection (Manassas, Va.).

**Ground pork and feces.** To assess initially the sensitivity of culture method, multiplex, and TaqMan PCR assays, ground pork was purchased at a local grocery store, irradiated at the Iowa State University Irradiation Facility, Ames, Iowa, and inoculated with *Y. enterocolitica* strain NADC 5561. Also, fecal samples were obtained from swine housed at the National Animal Disease Center, Ames, Iowa, autoclaved, and artificially inoculated with the same strain. *Y. enterocolitica* strain NADC 5561 was grown (37°C, overnight) with gentle shaking in 25 ml of LB broth. Tenfold serial dilutions of the overnight culture were made, and the bacterial concentration was determined by viable cell plate counts. From each bacterial dilution, 1-ml aliquots were inoculated into 1 g of ground pork or feces plus 8 ml of irgasan-ticarcillin-chlororate (ITC) (52) enrichment broth and incubated (48 h at room temperature). The estimated final concentrations ranged from $4 \times 10^2$ to $4 \times 10^7$ CFU/g ground pork or feces. Following enrichment (48 h, room temperature), 100-µl aliquots of the enrichments were plated to *Yersinia* selective agar (CIN agar, no. CM653; Oxoid, Hampshire, UK) supplemented with cefsulodin (15 µg/ml), irgasan (4 µg/ml), and novobiocin (2.5 µg/ml), and incubated (30°C, overnight). In addition, 1-ml aliquots from each sample were used to extract DNA by guanidine-silica particle extraction procedure and Prepman reagent. Uninoculated pork and fecal samples served as controls.

**Swine samples.** A total of 300 market weight swine (30 sampled each week) in the Midwest were screened for the presence of *Y. enterocolitica* over a 10-week interval. At the farm, feces and tonsil swabs were collected weekly from 30 randomly selected hogs. At slaughter tonsils, ileocecal, ventral thoracic, superficial inguinal lymph nodes, cecal and rectal contents, and carcass swabs were collected from the same hogs. Carcass swabs were taken from the ham and ventral surface of the carcasses with sponges (NASCO, Ft. Atkinson, Wis.) (48). All the samples were placed in sterile whirl packs (NASCO), transported to the laboratory on ice, and processed within 4 h of collection.

Tonsils and lymph nodes were homogenized in 25 ml of sterile buffered peptone water (pH 7.4) (no. CM509; Oxoid) in a Seward Stomacher 80 Lab System (1 min). Tissue homogenates (1 ml) and cecal and rectal contents (≈1 g each) were inoculated into 9 ml of ITC (52). The spleens were cut (3 by 1 by 0.5 cm) and aseptically placed in 9 ml of ITC. After enrichment (48 h at room temperature), 100-µl aliquots of ITC were plated to CIN agar and incubated (30°C, overnight), and plates were examined for the typical “bull’s eye” colonies of *Y. enterocolitica*. All colonies identified as presumptive *Y. enterocolitica* were verified using multiplex PCR.

**Pork products.** Seven trials were conducted using chitterlings and ground pork (50 samples per trial). Three trials were conducted with the pork products procured the same day of slaughter from a Midwest packing plant (plant A) as tissues from swine described above. Three trials included the pork products received from two major packing plants in the United States and are referred to as B (California) and C (Pennsylvania). One trial analyzed the pork products purchased from a retail grocery store in Alabama (D). Chitterlings were homogenized to form a paste, and 25 ml of this product was inoculated into 225 ml of ITC and subcultured to CIN as described above. Ground pork (25 g) was inoculated into 225 ml of ITC.

**Genomic DNA extraction.** Template DNA was extracted using a guanidine-silica particle extraction procedure (procedure 1), based on the DNA purification protocol of Carter and Milton (7). In short, a 1-ml aliquot of each ITC enrichment sample was pelleted (1 min, 14,000 × *g*). The supernatant was discarded, and the pellet was resuspended in 0.5 ml of DNA binding solution (1% diatomaceous earth in 6 M guanidine hydrochloride) and frozen (−80°C). The mixture was thawed for cell lysis and centrifuged (1 min, 14,000 × *g*); then, the pellet was washed twice with 70% ethanol. Following the second wash, the mixture was centrifuged (30 s, 14,000 × *g*), and the supernatant was aspirated.
The pellet was then resuspended in 50 μl of sterile water and heated (65°C for 10 min). The diatomaceous earth was then pelleted (1 min, 14,000 g, 15 s); and a 5' -3' extension (72°C, 500 mM KCl, pH 8.3), 200 μM dNTPs, 300 nM of each primer, and 1.25 U Taq polymerase (Boehringer Mannheim, Indianapolis, Ind.). The reaction profile was as follows: DNA denaturation (94°C, 3 min) prior to amplification; 40 cycles of denaturation (95°C, 10 s); primer annealing (56°C, 15 s); primer extension (72°C, 15 s); and final extension (72°C, 3 min).

Multiplex PCR. Oligonucleotide primers, based on the published sequence of the ail (33) and yadA (47) genes, were used in this study. The primers were commercially synthesized (Integrated DNA Technologies, Coralville, Iowa). The ail-specific primers generate a 425-bp product (15), and the yadA-specific primers generate a 308-bp product (54) (Table 1). The multiplex PCR was performed in a Gene Amp PCR system 9700 (Applied Biosystems) thermocycler. Each reaction (25 μl) contained: DNA template (3 μl), 10 times PCR reaction buffer (100 mM Tris-HCl, 15 mM MgCl₂, 500 mM KCl, pH 8.3), 200 μM dNTPs, 300 nM of each primer, and 1.25 U Taq polymerase (Boehringer Mannheim, Indianapolis, Ind.).

RESULTS

The specificity of both the assays was shown using purified DNA of non-Y. enterocolitica strains (Y. aldovae, Y. bercovieri, Y. frederiksenii, and Y. kristensenii) (Fig. 3). Neither of the assays amplified non-Y. enterocolitica DNA.

Detection limits for artificially inoculated pork and feces. Ground pork and feces were inoculated with 10-fold serial dilutions of known concentrations of Y. enterocolitica strain NADC 5561 and were enriched in ITC (48 h). For ground pork, the lower limit of detection was 4 × 10² CFU/g using the culture method. When DNA was extracted by guanidine-silica particle extraction protocol (procedure 1), the lower limits of detection were equivalent to 4 × 10³ CFU/g and 4 CFU/g for multiplex PCR and TaqMan assay, respectively. When Prepman DNA extraction protocol (procedure 2) was used, the lower limits of detection corresponded to 4 × 10³ CFU/g and 0.4 CFU/g for multiplex PCR and TaqMan assay, respectively (Fig. 4). For feces, the lower limit of detection was 4 × 10² CFU/g by multiplex PCR and 4 × 10⁵ CFU/g by TaqMan assay. Using DNA extraction procedure 2, the lower limit of detection was 4 × 10² CFU/g by multiplex PCR and 0.4 CFU/g by TaqMan assay (Fig. 4). Hence, the commercially available procedure 2 improved detection of the target sequences by one order of magnitude.
FIGURE 1. Specificity of ail- and yadA-specific primer pairs to amplify 425- and 308-bp fragments, respectively. DNA from Y. enterocolitica strains NADC 5561 and 5233 were used as a template in the multiplex PCR assay. Amplified products were analyzed on a 2% agarose gel. Lanes: M, digoxigenin-labeled pBR328 DNA cleaved separately with BglII and HinfI served as molecular weight standard; 1, multiplex PCR product using DNA from strain NADC 5561 as template; 2, no-template control; and 3, PCR product using DNA from strain NADC 5233. Arrowheads on the right side of the gel indicate the relative position of the two predicted PCR products, ail (425 bp) and yadA (308 bp).

Swine samples. As summarized in Table 2, a total of 2,403 swine specimens, including feces and tonsil swabs collected at the farm, and postslaughter samples (ileocecal, superficial inguinal, and thoracic lymph nodes; carcass swabs; and cecal and rectal contents) collected at the abattoir were screened for the presence of Y. enterocolitica using the three different methods. Y. enterocolitica was not detected in either of the pre- or postslaughter collections using the culture method (Table 2 and Fig. 5). Y. enterocolitica was detected in the preslaughter (1.5%) and in the postslaughter specimen (2.1%) using multiplex PCR. The overall prevalence of Y. enterocolitica as indicated by the TaqMan assay was 28 and 27% of the pre- and postslaughter specimens, respectively. Prevalence of Y. enterocolitica varied from tissue to tissue and was highest in the ileocecal lymph nodes according to both the multiplex PCR (7%) and TaqMan assays (39.9%). The lowest level of contamination was in the thoracic lymph nodes, as estimated by the multiplex PCR (0.8%) and TaqMan assays (14.6%) (Table 2 and Fig. 5). For swine specimens, using Cochran’s Q test for correlated proportions, there was no significant difference between culture versus multiplex PCR ($P < 0.07$), but there was a significant difference between culture versus TaqMan and multiplex versus TaqMan ($P < 0.01$). Again using the same test, there was no significant difference in the detection of Y. enterocolitica between farm fecal samples and rectal contents at slaughter ($P > 0.7$), but there was a significant difference between tonsil swabs and tonsil homogenates ($P < 0.01$).

Pork products. Chitterlings and ground pork samples (350 each) were screened for Y. enterocolitica using culture method, multiplex, and TaqMan PCR assays. Nineteen percent of the chitterling samples appeared positive when examined for the typical colonies of Y. enterocolitica. However, when the presumptive Y. enterocolitica colonies were verified using the multiplex PCR, only 8% were scored as positive for Y. enterocolitica (Table 3 and Fig. 6). When the multiplex PCR assay was used to screen the enrichments directly, 27% of chitterling samples were positive for Y. enterocolitica, while the TaqMan assay indicated a 79% prevalence. In all, there was a significant difference between culture versus multiplex ($P < 0.05$), culture versus TaqMan, and multiplex versus TaqMan ($P < 0.01$) using the Cochran’s Q test for correlated proportions. The detection of Y. enterocolitica was highest (18% by culture, 60% by multiplex, and 88% by TaqMan assay) in the chitterling samples prepared the same day of slaughter as the abovementioned swine (source A). Applying Fisher’s exact test for proportions ($P < 0.01$), there was a significant difference in the detection of Y. enterocolitica between sources A and B, C, and D for all the detection methods used. Sources B, C, and D were not significantly different ($P > 0.5$). Contamination (0% by culture, 0% by multiplex, and 68% by TaqMan assay) was lowest in chitterlings purchased from the retail grocery store (D) (Table 3).

Using the culture method, 7.1% of the ground pork samples appeared positive based on the presence of bull’s eye colonies, but none of the presumptive colonies were positive when screened by multiplex assay (Table 4 and Fig. 6). The overall prevalence of Y. enterocolitica as indicated by multiplex PCR and TaqMan assay for ground pork was 10 and 38%, respectively. There was no significant difference between culture versus multiplex ($P < 0.08$), but there was a difference between culture versus TaqMan and multiplex versus TaqMan ($P < 0.01$). For individual sources, the detection of Y. enterocolitica was highest (23% by multiplex and 53% by TaqMan) in the ground pork procured the same day of slaughter (source A), while ground pork purchased from the retail grocery store (source D) was 0% by all the assays (Table 4). Using Fisher’s exact test for proportions, there was no significant difference between the sources for culture ($P > 0.99$). For multiplex, source A (Midwest slaughterhouse) differed significantly from the other three sources ($P < 0.01$). For TaqMan, sources A and B (California slaughterhouse) were similar ($P > 0.09$), while all other pairs of sources were different ($P < 0.01$).
FIGURE 2. Sensitivity of the TaqMan assay for Y. enterocolitica. DNA isolated from Y. enterocolitica strain NADC 5561 was used as a template in the TaqMan assay in quantities of 100 ng/µl (sample 1), 10 ng/µl (sample 2), 1 ng/µl (sample 3), 100 pg/µl (sample 4), 10 pg/µl (sample 5), and 1 pg/µl (sample 6). Sample 7 is a no-template control. (A) The graph depicts the amplification for each template (left side indicates ΔRn, and right side indicates sample number); sample 1 is the 100-ng/µl template, etc. The threshold is defined as 10 times the standard deviation of the normalized fluorescent emission of the no-template control reactions. (B) Gel detection of the PCR products showing the 425-bp product of the ail gene. Lane M, 0.15 to 2.1 kbp DNA ladder. Lane 1 corresponds to the 100-ng/µl template (sample 1), etc.; lane 7 is the no-template control (sample 7).

DISCUSSION

Using purified Y. enterocolitica (NADC 5561) DNA, the TaqMan assay was 1,000 times more sensitive than conventional PCR assay in detecting ail-bearing strains. The TaqMan assay detected picogram quantities of DNA, compared to gel-based PCR assays that require 1 ng or more of DNA to generate a visually detectable band in ethidium bromide stained gels. We have reported that the TaqMan assay was able to detect between 0.25 and 0.5 pg of purified Y. enterocolitica DNA and that it did not cross-react with any of the 21 non-Y. enterocolitica strains examined (24). Previously, Wesley and Johnson (53) evaluated the specificity of conventional PCR assay using ail-specific primers and a variety of non-Y. enterocolitica species.

The results from pork and feces experimentally inoculated with Y. enterocolitica showed that the culture procedure was less sensitive than the PCR assays. For artificially contaminated pork, multiplex PCR was 10 times more sensitive than culture, while TaqMan assay was 100 to 1,000 times more sensitive than multiplex PCR and 10,000 times more sensitive than the culture method. For feces, the culture method was as sensitive as the multiplex PCR, while the TaqMan assay was 1,000 to 10,000 times more sensitive than the multiplex PCR or the culture procedure. We have reported that the TaqMan assay detected ≤1 CFU ail-bearing Y. enterocolitica per g of ground pork or feces after 24 h of enrichment in a Yersinia selective broth (24). In this study, the sensitivity of the PCR assays improved 10 to 100 times with DNA extraction procedure 2, which was optimized at the end of field sampling.
FIGURE 3. Specificity of the multiplex PCR and TaqMan assays for ail-bearing Y. enterocolitica. Non-Y. enterocolitica strains (100 ng/μl each) Y. aldovæ: NADC 5612, Y. bercovieri: NADC 5615, Y. frederiksenii: NADC 5617, and Y. kristensenii: NADC 5622 were used to test the specificity of both assays. (A) The plots show no amplification exceeding the threshold. (B) Agarose gel electrophoresis image shows no amplification of the non-Y. enterocolitica species (lanes 1 through 4). The arrow indicates the position of the ail gene. Lane M, 0.15 to 2.1 kbp DNA ladder; lane 1, Y. aldovæ; lane 2, Y. bercovieri; lane 3, Y. frederiksenii; and lane 4, Y. kristensenii.

It was observed that swine and pork samples that showed the highest ΔRn values (3.9 to 5.8) using the TaqMan assay were also positive using the multiplex PCR assay. In contrast, those samples with lower ΔRn values (below 3.5 but above the threshold value) were scored positive by TaqMan but were negative by conventional gel detection, further reflecting the sensitivity of the assay over multiplex. The NADC 5233 isolate was amplified at a lower efficiency (lower ΔRn) than NADC 5561 in the TaqMan assay (with the same DNA concentration per reaction). This is in agreement with the appearance of faint bands (NADC 5233) using conventional PCR. Beer and Miller (4) have shown that there are at least two ail gene variants between different Y. enterocolitica strains. Therefore, it is possible that the sequence of ail targeted in the assays varies slightly between different strains, which account for the inconsistencies in amplification and fluorescence emission (24). Moreover, Kapperud et al. (25) reported that in a two-step PCR assay using yadA-specific primers, the PCR products exhibited small size variations depending on the serogroup affiliation of the test strain.

None of the pre- or postslaughter swine samples or ground pork samples yielded Y. enterocolitica by culture; by contrast, 8% of chitterling samples were positive. This study implies that the contamination level in swine at farm and postslaughter is <4 × 10^3 CFU/g, which was the detection limit for the culture method. Colonies with the same appearance as pathogenic Y. enterocolitica bacteria may outcompete this microbe and make it difficult to select the appropriate colonies for confirmation (51). Therefore, it is possible that conventional culture methods underestimate the prevalence of ail-bearing strains of Y. enterocolitica in clinical and food samples.
TABLE 2. Detection of \textit{Y. enterocolitica} in swine

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Samples analyzed ((n = 2,403))</th>
<th>Number of positive isolates (% of samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture(^a)</td>
<td>Multiplex PCR</td>
</tr>
<tr>
<td>Preslaughter Fecal sample</td>
<td>297</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tonsil swabs</td>
<td>297</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ileocecal LN</td>
<td>257</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tonsils</td>
<td>252</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Rectal contents</td>
<td>255</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cecal contents</td>
<td>257</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Carcass swabs</td>
<td>267</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Thoracic LN</td>
<td>259</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Superficial inguinal LN</td>
<td>262</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>2,403</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

\(^a\) Presumptive colonies were verified using multiplex PCR.

\textit{Y. enterocolitica} was detected more frequently in artificially inoculated pork, hog specimens, and pork products by multiplex PCR than by the culture method. Previously, Fredriksson-Ahooma et al. (18) showed that the prevalence of \textit{Y. enterocolitica} carrying the \textit{yadA} gene was 92% in pig tongues and 25% in minced meat using PCR and 78% in tongues and 2% in minced meat with the culture method. Likewise, the detection rates of \textit{yadA}-positive \textit{Y. enterocolitica} for livers (38 and 31%), kidneys (86 and 69%), and hearts (63 and 50%) were higher using PCR versus culture (19). One of the significant problems is that PCR does not differentiate between viable and nonviable cells. However, in this study, the introduction of enrichment step favored the detection of viable cells (2). In addition, any DNA released from dead microbes is rapidly degraded.

There was no significant difference (\(P > 0.66\)) between the detection in feces and tonsil swabs. This suggests that screening one site may be adequate to determine the prevalence of \textit{Y. enterocolitica} in the live animals, thus eliminating the need to collect both fecal and tonsil swab samples. In general, \textit{Y. enterocolitica} is detected more frequently in tonsil swabs than in feces; thus, field surveys may include only tonsil swabs (9). For example, Rasmussen et al. (42) reported that 40% of fecal samples and 84% of tonsil swab samples were positive for \textit{Y. enterocolitica}. Nielsen and Wegener (36) reported that \textit{Y. enterocolitica} persists longer in tonsils of experimentally infected pigs than in feces.

The detection of \textit{Y. enterocolitica} from tonsil homogenates was statistically higher when compared to its detection from tonsil swabs, based on Cochran’s Q test for correlated proportions (\(P < 0.01\)). Previously, Fredriksson-Ahooma et al. (18) demonstrated that 69% of the tongues were positive for \textit{Y. enterocolitica} when tissue samples were used and that only 37% were positive when swab samples were used. For practical purposes, antemortem on farm testing must rely on tonsil scraping or, at best, tonsillar biopsy.

Among all of the swine specimens screened, \textit{Y. enterocolitica} was detected most frequently in the ileocecal lymph nodes both by multiplex PCR (7%) and TaqMan assay (39.9%), indicating that the organism localizes in the ileocecal region of the gut of the swine (32). Thoracic (0.8%, 14.6%) and superficial inguinal lymph nodes (1.1%,

TABLE 3. Detection of \textit{Y. enterocolitica} in chitterlings

<table>
<thead>
<tr>
<th>Source (packing plant)</th>
<th>Samples analyzed ((n = 350))</th>
<th>Number of positive isolates (% of samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture(^a)</td>
<td>Multiplex</td>
</tr>
<tr>
<td>A</td>
<td>50</td>
<td>16 (32)</td>
</tr>
<tr>
<td>A</td>
<td>50</td>
<td>0 (0)</td>
</tr>
<tr>
<td>A</td>
<td>50</td>
<td>11 (22)</td>
</tr>
<tr>
<td>Total (A)</td>
<td>150</td>
<td>27 (18)</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>0 (0)</td>
</tr>
<tr>
<td>B</td>
<td>50</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Total (B)</td>
<td>100</td>
<td>1 (1)</td>
</tr>
<tr>
<td>C</td>
<td>50</td>
<td>0 (0)</td>
</tr>
<tr>
<td>D</td>
<td>50</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total (A, B, C, D)</td>
<td>350</td>
<td>28 (8)</td>
</tr>
</tbody>
</table>

\(^a\) Presumptive colonies were verified using multiplex PCR.
FIGURE 5. Comparison of culture, multiplex PCR, and TaqMan assays in detecting Y. enterocolitica from swine. (A) The recovery rate from preslaughter specimens, including feces and tonsils. (B) Detection from postslaughter specimens, including tonsils, lymph nodes, carcass swabs, and cecal and rectal contents.

17.9%) were included in this study because they are the components of edible meat. The carcass swabs were found positive by multiplex PCR (2%) and by TaqMan assay (27.3%). Previous studies reported that high contamination rates of Y. enterocolitica in the pork might be due to the contamination of carcasses by infected tonsils, tongues, and gut contents at slaughter (1, 18, 52).

The prevalence of Y. enterocolitica in chitterlings was significantly higher than in ground pork for the three screening methods. Lee et al. (29) reported that more than

FIGURE 6. Detection of Y. enterocolitica from chitterlings and ground pork using three detection assays.
half of chitterling containers sampled were positive for *Y. enterocolitica*. Schiemann (43) isolated *Y. enterocolitica* from 48% of 128 raw pork products obtained from retail stores in Canada. In our study, the detection of *ail*-bearing strains was fairly low in the ground pork purchased in the retail grocery store (D), when compared to the detection in the samples received from the packing plants on six occasions. De Giusti et al. (12) showed that none of the *Yersinia* spp. were detected in retail pork products by any of the procedures used, while Fukushima (20) reported that, out of 125 samples of retail ground pork, six (4.8%) contained *Y. enterocolitica* by the direct isolation method. It is possible that such differences are due to the variations in the prevalence among the pig herds.

The TaqMan assay indicated the presence of *ail*-bearing *Y. enterocolitica* in 27.2% (*n* = 2,403) of the swine samples, 79% (*n* = 350) of the chitterling samples, and 38% (*n* = 350) of the ground pork samples. However, quantitation of the contamination levels was not attempted, although the TaqMan assay allows for the enumeration of starting material present in a given sample. Interestingly, when the fluorescence signal was observed for artificially inoculated pork and feces, 4 × 10^5 and 4 × 10^4 CFU/g of pork generated a positive signal between 13 and 16 amplification cycles, while for feces, the signal was observed between 15 and 18 cycles by the TaqMan assay. When randomly selected samples of the ground pork and farm feces (50 each) were analyzed, the positive signal was generated later between 24 and 33 amplification cycles. The infective dose of *Y. enterocolitica* for humans is likely to exceed 10^4 CFU (17). Accordingly, not all the positive samples obtained in this study pose a significant public health risk. Further, the quantitative estimation of the cells present in an individual sample is essential to assess the public health significance of finding *Y. enterocolitica*.

In conclusion, the TaqMan assay appears to be 1,000 to 10,000 times more sensitive than multiplex PCR and even more sensitive than the culture method in detecting *ail*-bearing *Y. enterocolitica* in food matrices. Moreover, the TaqMan assay provides for a complete automated and high-throughput screening of a variety of samples for the rapid detection of *Y. enterocolitica*. These rapid detection methods may be used to evaluate hazard analysis and critical control points protocols for the reduction of potential foodborne pathogens in pork (13).

**ACKNOWLEDGMENTS**

We thank Dr. Alissa D. Jourdan for her technical guidance. We also thank Dr. Harold D. Ridpath for providing statistical analysis of the data and Brad O. Chriswell for providing untried and enthusiastic technical assistance in this study. We gratefully acknowledge Dr. Vijay Sharma, Dr. Jonathan B. Katz, and Dr. Tim Frana for their helpful comments on the manuscript. This research was partially supported by the Iowa State University Food Safety Consortium and the National Pork Producers Council.

**REFERENCES**


