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# Prevalence of Pathogenic *Yersinia enterocolitica* Strains in Pigs in the United States

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***Yersinia enterocolitica* is considered an important food-borne pathogen impacting the pork production and processing industry in the United States. Since this bacterium is a commensal of swine, the primary goal of this study was to determine the prevalence of pathogenic *Y. enterocolitica* in pigs in the United Sates using feces as the sample source. A total of 2,793 fecal samples were tested for its presence in swine. Fecal samples were collected from late finisher pigs from 77 production sites in the 15 eastern and midwestern pork-producing states over a period of 27 weeks (6 September 2000 to 20 March 2001). The prevalence of *ail*-positive *Y. enterocolitica* was determined in samples using both a fluorogenic 5′ nuclease PCR assay and a culture method. The mean prevalence was 13.10% (366 of 2,793 fecal samples tested) when both PCR- and culture-positive results were combined. Forty-one of 77 premises (53.25%) contained at least one fecal sample positive for the *ail* sequence. The PCR assay indicated a contamination rate of 12.35% (345/2,793) compared to 4.08% (114/2,793) by the culture method. Of the 345 PCR-positive samples, 252 were culture negative, while of the 114 culture-positive samples, 21 were PCR negative. Among 77 premises, the PCR assay revealed a significantly ($P < 0.05$) higher percentage (46.75%, $n = 36$ sites) of samples positive for the pathogen (*ail* sequence) than the culture method (22.08%, $n = 17$ sites). Thus, higher sensitivity, with respect to number of samples and sites identified as positive for the PCR method compared with the culture method for detecting pathogenic *Y. enterocolitica*, was demonstrated in this study. The results support the hypothesis that swine are a reservoir for *Y. enterocolitica* strains potentially pathogenic for humans.**

*Yersinia enterocolitica* is a food-borne pathogen estimated to cause 96,000 cases of human disease annually in the United States (15, 16). Ninety percent of those cases are the result of food-borne transmission (16). Swine are the primary reservoir from which *Y. enterocolitica* strains pathogenic to humans are isolated, and the organism is a major bacterial food-borne pathogen by the pork production and processing industry in the United States (7). In countries where *Y. enterocolitica* is an important food-borne pathogen, the prevalence of *Y. enterocolitica* in the swine population is well documented (15, 16). Estimates of pathogenic *Y. enterocolitica* carriage in swine range from 35 to 70% of herds and 4.5 to 100% of individual swine.

In the United States, few studies on the prevalence of *Y. enterocolitica* in swine have been conducted. Lee et al. (14) reported more than half of containers of chitterlings sampled, originating from pigs slaughtered in different regions of United States, were positive for *Y. enterocolitica*. In a case control study of *Y. enterocolitica* infections among black infants, chitterling preparation was significantly associated with illness (11). Recently, in the United States, using a real-time PCR format, Boyapalle et al. (6) reported the presence of pathogenic *Y. enterocolitica* from swine as well as in pork. Funk et al. (10) identified *Y. enterocolitica* from oral-pharyngeal samples (13.2%) of pigs at slaughter. Bhaduri et al. (5) and Bhaduri and Cottrell (3, 4) detected and isolated plasmid-bearing virulent *Y. enterocolitica* in 41% of pork tongues. Kotula and Sharer (13) also isolated *Y. enterocolitica* from 4% of cecal contents of 50 pigs at slaughter.

Virulent strains of *Y. enterocolitica* which are pathogenic to humans harbor the *ail* gene, which encodes for attachment-invasion protein (16). A commonly used method, which has relatively high sensitivity and specificity for detecting pathogenic *Y. enterocolitica* in swine, pork products, and feces is based on the PCR targeting the *ail* gene (4, 12, 19). A fluorogenic 5′ nuclease PCR assay targeting the *ail* gene allowed automated PCR amplification, detection, and analysis of pathogenic *Y. enterocolitica* in ground pork and pig feces. The specificity and sensitivity of this assay were estimated to be 100% for detection of this organism (6, 12).

In pigs, *Y. enterocolitica* is spread by the fecal oral route (1, 17). Since pork safety begins on the farm, producers and practitioners play a critical role in providing safe products for United States and international consumers. Thus, there is a need to monitor swine for *ail*-harboring virulent strains of *Y. enterocolitica*. Once a baseline is established, continuous monitoring may be used to assess the effectiveness of on-farm pathogen reduction programs. Therefore, a study was conducted as a part of the United States Department of Agriculture's (USDA) National Animal Health Monitoring System

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(NAHMS) Swine 2000 Study established by USDA's Animal and Plant Health Inspection Services (APHIS) to determine the prevalence of pathogenic *Y. enterocolitica* in swine. The goals of this study were to measure the degree to which food-borne pathogens are found in finisher pigs and to identify potential control factors to reduce the public health risk from these pathogens (18). This study included on-farm sampling which canvassed 1,000 operations from the top 15 hog-producing states. This operation represented 92% of the United States hog inventory and 75% of its operations.

Since this bacterium is a commensal of swine, the primary goal of this study was to identify the prevalence of pathogenic *Y. enterocolitica* in fecal samples of market pigs using fluorogenic 5′ nuclease PCR assay and a culture method. The information generated will be useful for the identification of on-farm management and processing practices leading to *Y. enterocolitica* contamination. Modification of such practices would ultimately result in reducing *Y. enterocolitica* transmission from pork products to humans.

## MATERIALS AND METHODS

**Field collection.** On-farm sampling was conducted from 6 September through 6 December 2000 and from 3 January through 20 March 2001. Fecal pen floor samples were collected on farms ($n = 77$) from 15 of the top 17 pork-producing states including, in alphabetical order, Arizona, Colorado, Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Carolina, Ohio, Pennsylvania, South Dakota, and Wisconsin. A total of 2,793 fecal samples (up to 50 samples per farm) were collected from late finisher pigs for testing for the presence of pathogenic *Y. enterocolitica*. The proportion of samples collected from each state reflected that state's contribution to the overall national swine population. On-farm collections were performed by APHIS federal and state field veterinarians. The samples were shipped in styrofoam coolers containing freezer packs. The interval from sample collection on farms to sample analysis in our laboratory at Eastern Regional Research Center was between 48 and 72 h.

**Preparation of media.** Irgasan, ticarcillin, potassium chlorate broth (ITC) was prepared according to Wauters et al. (20) as follows: 10 g tryptone, 1 g yeast extract, 60 g $MgCl_2 \cdot 6H_2O$, 5 g NaCl, 1 g $KClO_3$, and 5 ml of 0.2% malachite green were added to 1 liter of water. After sterilization, Timentin (sterile ticarcillin disodium and clavulanate potassium; SmithKline Beecham Pharmaceuticals, Philadelphia, PA) and Irgasan DP 300 (Ciba-Geigy, Basel, Switzerland) were each added at a final concentration of 1 μg/ml. Brain heart infusion (BHI) broth, brain heart infusion agar (BHA), and 0.1% peptone water (Difco Laboratories, Detroit, MI) were prepared as recommended by the manufacturer, as was cefsulodin-irgasan-novobiocin (CIN; Oxoid, Unipath Ltd., Basingstoke, Hampshire, England).

**Enrichment of fecal samples.** One gram from each fecal sample was added to a Whirl Pak stomacher bag (Nasco, Ft. Atkinson, WI), suspended in 9 ml of 0.1% peptone water, and then mixed in a Stomacher 80 laboratory blender (Seward Medical, London, England) for 30 s. One milliliter of the suspension was added to 9 ml of ITC broth in a 50-ml centrifuge tube and briefly vortexed. The enrichment was held at room temperature (25°C) for 48 h.

**Sampling of the enrichments.** The enrichments were vortexed and then centrifuged in a microcentrifuge (425 relative centrifugal force [RCF]) for 30 s (model 5810R; Eppendorf, Hamburg, Germany) to remove heavy particulates. A 1.5-ml aliquot of enriched culture from each sample was plated to CIN for the isolation of *Y. enterocolitica*. Also, 1.0 ml of each enriched culture was placed in a 1.5-ml Eppendorf microfuge tube and pelleted (16.1 K RCF for 2 min, Eppendorf model 5415D). The supernatant was aspirated, and the pellet was washed twice with TE buffer (10 mM Tris-HCl–0.1 mM EDTA, pH 7.2) and used for DNA extraction.

**DNA extraction.** DNA extraction was performed by using PrepMan reagent (PE Applied BioSystems, Foster City, CA) according to the manufacturer's instructions. Briefly, PrepMan reagent (200 μl) was added to each sample and vortexed to suspend the pellet. The samples were lysed (100°C for 10 min) in a water bath and chilled on ice. The samples were pelleted (20.8 K RCF for 2 min, Eppendorf model 5810R). One hundred microliters of the supernatant was transferred to a new 1.5-ml centrifuge tube and served as the template in the PCR assay to detect pathogenic *Y. enterocolitica*.

**Fluorogenic 5′ nuclease PCR assay for detection of pathogenic *Y. enterocolitica* strains in swine feces.** The fluorogenic 5′ nuclease PCR assay was performed essentially as described by Jourdan et al. (12). DNA template was added to a PCR containing primers amplifying an *ail* gene sequence from *Y. enterocolitica.* The presence of the *ail* gene specifically differentiates pathogenic from environmental strains of *Y. enterocolitica* lacking this gene (1, 16). The primers targeting the *ail* gene sequences (forward, 5′-GGTCATGGTGATGTTGATTACTATTC A-3′); (reverse, 5′-CGGCCCCCAGATATACCATA-3′) were commercially synthesized (Integrated DNA Technologies, Inc., Coralville, IA) and amplified a 118-bp DNA fragment. Detection employed a 5′ FAM (6-carboxyfluorescein) and 3′ TAMRA (6-carboxytetramethylrhodamine) fluorescently labeled reporter probe having the sequence 5′-CATCTTTCCGCATCAACGAATATGTTAG C-3′ (Synthetic Genetics, San Diego, CA). The PCR mixture contained 1× GeneAmp PCR Gold buffer (15 mM Tris-HCl [pH 8.0], 50 mM KCl; PE Applied Biosystems, Foster City, CA), 3.5 mM $MgCl_2$ (PE Applied Biosystems), 0.2 mM deoxynucleoside triphosphates (PE Applied Biosystems), 200 nM primers, 25 nM probe, 1.25 U/μl Ampli*Taq* Gold DNA polymerase (PE Applied Biosystems), and 5 μl template. Thermal cycling conditions were as follows: 95°C for 10 min, followed by 35 cycles of 95°C for 15 s and 58°C for 1 min, followed by an indefinite hold at 25°C. PCR was performed in a 96-well format using a PerkinElmer 9600 thermal cycler (PE Applied Biosystems) and detection employed an ABI Prism 7200 sequence detector (PE Applied BioSystems) with Sequence Detector software version 1.6.3 (PE Applied Biosystems). Data analysis was performed against no-template controls to determine positive samples (99% confidence level).

**Isolation of presumptive *Y. enterocolitica* colonies.** The enriched cultures were vortexed, diluted 1:10 in 0.1% peptone water to reduce background contaminating flora and a 100-μl aliquot plated on CIN agar for presumptive isolation. All plates were incubated at 28°C for 24 h. *Y. enterocolitica* formed small (~1- to 2-mm-diameter) colonies with a deep red center and a sharp border surrounded by a clear colorless zone. A maximum of five colonies per plate were subcultured to BHA at 28°C for 24 h. A portion of the bacterial growth was suspended in 1 ml of TE buffer for identification of pathogenic *Y. enterocolitica* strain and the remainder stored.

**Confirmation of CIN-positive presumptive clones as pathogenic strains by fluorogenic 5′ nuclease PCR assay.** DNA was extracted from CIN-positive presumptive *Yersinia* colonies as described previously and was subjected to a fluorogenic 5′ nuclease PCR assay (12) for confirmation of pathogenic *Y. enterocolitica* strains on the basis of amplification of the chromosomal *ail* gene.

**Storage of pathogenic *Y. enterocolitica* strains recovered from swine feces.** The PCR-positive pathogenic isolates were picked using a sterile needle and were then cultured in 5 ml BHI broth for 18 to 24 h at 28°C. The cells were harvested and washed with 5 ml of BHI broth. The cell pellet was resuspended in 2.5 ml of BHI broth and then 2.5 ml of BHI broth with 20% glycerol was added to give a final glycerol concentration of 10%. Portions (200 μl) were aliquoted into cryogenic vials and stored (−70°C).

**Statistical analysis.** The data from individual samples and from the sites were analyzed to test the agreement between the two methods of detection. The positive detection rates of the PCR and culture methods were compared using McNemar's test (8).

## RESULTS AND DISCUSSION

Swine fecal samples ($n = 2,793$) from 77 production sites were surveyed for the presence of pathogenic *Y. enterocolitica* over a period of 27 weeks (6 September 2000 to 6 December 2001 and 3 January through 20 March 2001) (Table 1). The sites were located in 2 eastern and 13 midwestern states. Kit number for each site, date collected and numbers of fecal samples per collected site are shown in Table 1. A fluorogenic 5′ nuclease PCR assay (12) detected the chromosomal *ail* gene in 345 of 2,793 enrichments, some of which were subsequently culture positive ($n = 93$) (Table 2), although the majority were culture negative ($n = 252$) (Table 2). In addition, CIN-positive colonies which were negative by the fluorogenic 5′ nuclease PCR assay ($n = 21$) were identified. Thus, a combined total of 366 ($n = 93 + 252 + 21$) cultures (13.10%) from 2,793 fecal samples contained pathogenic *Y. enterocolitica* by either of these two techniques (Table 2). As summarized in Tables 1 and

TABLE 1. Detection and isolation of pathogenic *Y. enterocolitica* from swine feces

| Kit[a] no. | Date (mo/day/yr) collected | No. tested | PCR[b] positive | Culture[c] positive |
|---|---|---|---|---|
| 6 | 9/6/00 | 56 | 1 | 0 |
| 2 | 9/18/00 | 25 | 0 | 0 |
| 30 | 9/18/00 | 5 | 0 | 0 |
| 35 | 9/19/00 | 50 | 0 | 0 |
| 14 | 9/20/00 | 27 | 0 | 0 |
| 34 | 9/20/00 | 25 | 0 | 0 |
| 20 | 10/2/00 | 13 | 0 | 0 |
| 21 | 10/2/00 | 13 | 0 | 0 |
| 33 | 10/2/00 | 19 | 0 | 0 |
| 37 | 10/2/00 | 11 | 0 | 0 |
| 1 | 10/2/00 | 25 | 0 | 0 |
| 23 | 10/10/00 | 50 | 4 | 0 |
| 62 | 10/10/00 | 43 | 4 | 0 |
| 61 | 10/16/00 | 45 | 21 | 0 |
| 66 | 10/16/00 | 5 | 3 | 0 |
| 26 | 10/17/00 | 40 | 1 | 0 |
| 53 | 10/16/00 | 7 | 0 | 0 |
| 55 | 10/23/00 | 50 | 0 | 2 |
| 19 | 10/25/00 | 9 | 0 | 0 |
| 17 | 10/25/00 | 50 | 0 | 0 |
| 56 | 10/24/00 | 50 | 0 | 0 |
| 52 | 10/30/00 | 20 | 6 | 0 |
| 78 | 10/30/00 | 25 | 9 | 0 |
| 44 | 10/31/00 | 25 | 4 | 0 |
| 59 | 10/31/00 | 25 | 1 | 0 |
| 74 | 10/31/00 | 15 | 0 | 0 |
| 76 | 11/6/00 | 50 | 0 | 0 |
| 79 | 11/1/00 | 42 | 1 | 0 |
| 77 | 11/8/00 | 30 | 0 | 0 |
| 84 | 11/28/00 | 50 | 0 | 0 |
| 87 | 11/27/00 | 19 | 0 | 0 |
| 72 | 11/28/00 | 35 | 0 | 0 |
| 94 | 12/4/00 | 50 | 12 | 0 |
| 100 | 12/5/00 | 30 | 2 | 1 |
| 45 | 12/6/00 | 50 | 17 | 0 |
| 16 | 1/3/01 | 41 | 4 | 0 |
| 89 | 1/3/01 | 50 | 0 | 0 |
| 110 | 1/4/01 | 50 | 0 | 1 |
| 135 | 1/17/01 | 50 | 12 | 0 |
| 104 | 1/16/01 | 50 | 6 | 1 |
| 116 | 1/16/01 | 36 | 0 | 3 |
| 46 | 1/17/01 | 50 | 0 | 0 |
| 114 | 1/17/01 | 38 | 9 | 2 |
| 70 | 1/18/01 | 33 | 3 | 0 |
| 128 | 1/18/01 | 14 | 0 | 0 |
| 123 | 1/29/01 | 49 | 1 | 0 |
| 127 | 1/29/01 | 40 | 7 | 0 |
| 122 | 1/29/01 | 50 | 10 | 3 |
| 129 | 1/29/01 | 50 | 2 | 2 |
| 117 | 1/29/01 | 27 | 11 | 0 |
| 119 | 1/30/01 | 50 | 0 | 1 |
| 118 | 1/31/01 | 50 | 4 | 0 |
| 139 | 1/30/01 | 50 | 42 | 7 |
| 142 | 2/5/01 | 55 | 0 | 0 |
| 132 | 2/6/01 | 25 | 0 | 0 |
| 115 | 2/7/01 | 20 | 14 | 16 |
| 141 | 1/12/01 | 30 | 14 | 10 |
| 144 | 2/12/01 | 53 | 3 | 0 |
| 140 | 2/14/01 | 50 | 0 | 0 |
| 145 | 2/12/01 | 58 | 0 | 0 |
| 124 | 2/12/01 | 50 | 0 | 0 |
| 85 | 2/14/01 | 50 | 0 | 0 |
| 137 | 2/6/01 | 25 | 0 | 0 |
| 50 | 2/15/01 | 50 | 3 | 0 |
| 146 | 2/27/01 | 50 | 0 | 0 |
| 111 | 2/27/01 | 50 | 18 | 15 |
| 49 | 2/26/01 | 21 | 11 | 1 |
| 81 | 2/28/01 | 50 | 0 | 12 |
| 82 | 2/26/01 | 50 | 0 | 0 |
| 90 | 2/28/01 | 60 | 0 | 0 |
| 143 | 2/28/01 | 30 | 21 | 0 |
| 7 | 2/28/01 | 52 | 15 | 10 |
| 149 | 3/6/01 | 50 | 47 | 27 |
| 102 | 3/19/01 | 13 | 0 | 0 |
| 150 | 3/20/01 | 13 | 0 | 0 |
| 151 | 3/20/01 | 13 | 2 | 0 |
| 152 | 3/20/01 | 13 | 0 | 0 |
| Total | | 2793 | 345 | 114 |

[a] Samples collected from one location on 1 day comprised one kit.
[b] Number of samples shown to possess the *ail* gene by PCR assay.
[c] Total number of isolates obtained from different fecal samples from one kit. Pathogenic *Y. enterocolitica* identified by plating on CIN agar and PCR assay on red to pink colonies.

2, the *ail* gene was detected by PCR in 345 out of 2,793 (12.35%) fecal samples whereas 114 pathogenic *Y. enterocolitica* clones were isolated from 2,793 fecal samples (4.08%) by subculture to CIN agar. Only 93 (3.33%) of samples from 2,793 fecal samples were positive by both techniques (Table 2). As shown in Table 2, of the positive samples, 9.02% (252 of 2,793) were positive only by PCR and only 0.75% of samples (21 of 2,793) were positive only by culture. Of the 114 culture-positive samples, although the majority (81.5% [93 of 114]) harbored the *ail* gene, this virulence factor could not be detected in 18% (21 of 114) of the CIN-positive cultures. That these 21 culture-positive samples were negative in the PCR assay might be due to the presence of inhibitors in the DNA template (2), to the true absence of the *ail* gene indicating that these strains were nonpathogenic, or to poor sensitivity of the real-time assay. Thus, on the basis of testing of pen floor fecal samples, an estimated 13.00% of hogs harbored *Y. enterocolitica*. Pathogenic strains were more frequently detected in feces with the PCR method (12.54%) compared to culture (4.10%). McNemar's test (8) showed that there was a significantly ($P < 0.05$) higher sensitivity with the PCR method.

TABLE 2. Frequency of results of positive and negative responses from fecal samples by the PCR and the culture techniques

| PCR results | Culture positive[b] | Culture negative | Row total |
|---|---|---|---|
| PCR positive[a] | 93 | 252 | 345 |
| PCR negative | 21 | 2,427 | 2,448 |
| Column total | 114 | 2,679 | 2,793 |

[a] Number of *ail*-positive feces samples detected in ITC enrichment by PCR assay.
[b] Number of *ail*-positive isolates confirmed by PCR assay in feces with the culture method.

In 41 of 77 production sites, at least one pig was positive for the *ail* sequence either by direct screening of the enrichment (*n*

TABLE 3. Frequency of results of positive and negative sites based on PCR and the culture techniques

| PCR results[a] | Culture positive[b] | Culture negative | Row total |
|---|---|---|---|
| Positive | 12 | 24 | 36 |
| Negative | 5 | 36 | 41 |
| Column total | 17 | 60 | 77 |

[a] Number of *ail*-positive sites by PCR assay of ITC enrichment.
[b] Number of culture-positive sites confirmed by PCR assay targeting the *ail* gene.

TABLE 4. Seasonal prevalence of pathogenic *Yersinia enterocolitica* in swine feces

| Duration | No. of samples | No. of positive samples[a] | % Positive samples |
|---|---|---|---|
| 6 September–6 December 2000 | 1,084 | 92 | 8.49% |
| 3 January–2 March 2001 | 1,709 | 274 | 16.03% |

[a] Number of *ail*-positive feces samples when both PCR assay and culture-positive results were combined.

= 36) or by culture alone ($n$ = 5), resulting in an overall 53.25% on-farm prevalence rate (Tables 1 and 3). Using fecal samples to monitor the hog carrier status, the on-farm prevalence based on the PCR assay alone was 46.75% (36 of 77 premises sampled, [Table 3]). Among the 41 positive sites, 17 sites showed a significantly higher percentage (20 to 94%) of pathogenic *Y. enterocolitica* by the PCR assay; six sites showed a significantly higher percentage (20 to 80%) of pathogenic *Y. enterocolitica* isolates by selective culture on CIN (Table 1). Twenty-four of 36 sites (31.17%) were positive only by the PCR assay (Table 3). The prevalence based on growth on CIN agar was 22.08% (17 of 77 premises [Tables 1 and 3]). Five (6.49%) of these 17 positive sites were positive only by the culture method (Table 3). Twelve sites (15.58%) were positive by both methods (Table 3). Overall, a significantly ($P < 0.05$) higher percentage of premises were positive by the PCR assay (36 of 77 premises [46.80%]) than by the culture method (17 of 77 premises, 22.07%). This indicates that the growth in ITC broth is more permissive as well as more suitable for pathogenic strains than selective CIN media and that selective plating may compromise the recovery of *Yersinia*. Both Tables 2 and 3 showed that more positive samples were identified by PCR than by the culture method. Thus, the PCR assay is more sensitive and data based solely on results obtained by plating onto CIN agar may underestimate the prevalence of pathogenic *Y. enterocolitica.* This is in agreement with Boyapalle (6) who concluded that the fluorogenic 5′ nuclease PCR assay was more sensitive than the culture method. That the presence of *Y. enterocolitica* in feces varied from site to site indicates risk factors that influence its presence on farms.

The results of this study support the hypotheses that swine represent a potential reservoir for *Y. enterocolitica* strains potentially pathogenic for humans. The percentage of production sites positive for pathogenic *Y. enterocolitica* (46.75% by the PCR assay and 22.08% by selective plating) was within the range of 26 to 50% estimated from previous studies (10). In this current study, the number of fecal samples positive (13.10%) for the *ail* gene (12.35% by the PCR assay and 4.08% by selective plating) was similar (13.00%) to that of Funk et al. (10) who examined oral-pharyngeal samples instead of feces. Bhaduri and Cottrell (3, 4) found that 41% of pork tongue samples were positive for pathogenic *Y. enterocolitica* by the PCR assay, Congo red binding, and low calcium response isolation techniques. Thus it may be more difficult to detect/isolate pathogenic *Y. enterocolitica* from feces compared to other types of samples from swine. The prevalence was clearly higher using the PCR method compared to the culture technique, suggesting higher sensitivity and rapidity of the PCR assay in analyzing swine fecal samples.

In abattoir studies, the seasonal occurrence of pathogenic *Y. enterocolitica* was tested for equal rates of occurrence by using the chi-squared test (8). Table 4 shows that the rate of detection/isolation of the organism was significantly higher ($P <$ 0.05) at a ratio of 1.89 from 3 January to 20 March 2001 (16.03%) than the rate from 6 September to 6 December 2000 (8.49%). Fukishima et al. (9) also observed that the frequency of detection/isolation of this organism from swine was higher during the cooler months of the year. The results of this study support the hypothesis that swine are a significant potential reservoir for *Y. enterocolitica* strains, which are pathogenic to humans. The varying presence of *Y. enterocolitica* from site to site suggests that management factors may influence on-farm prevalence of this organism. To fully understand the prevalence and risk factors associated with *Y. enterocolitica* infection in swine, more investigations are needed on farms and throughout production and processing systems.

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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 U.S. Department of Agriculture.

## ADDENDUM IN PROOF

A similar study on the prevalence of pathogenic *Y. enterocolitica* in fattening pigs (M. Gurtler, T. Alter, S. Kasimir, M. Linnebur, and K. Fehlhaber, J. Food Prot. **68:**850–854, 2005) was published while this study was being reviewed.

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