Testing of Swine Feces Obtained through the National Animal Health Monitoring System's Swine 2000 Study for the Presence of *Escherichia coli* O157:H7

INGRID FEDER,† JEFFREY T. GRAY,‡ RACHEL A. PEARCE,‖ PINA M. FRATAMICO,‖ ERIC BUSH,‖ ANNA PORTO-FETT,‖ F. MORGAN WALLACE,‖ PAULA J. FEDORKA-CRAY,‖ and JOHN B. LUCHANSKY

1U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, Pennsylvania 19038; 2U.S. Department of Agriculture, Agricultural Research Service, Richard B. Russell Research Center, 950 College Station Road, Athens, Georgia 30605; and 3U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Center for National Animal Health Surveillance, 555 South Howes Street, Fort Collins, Colorado 80526, USA

MS 06-598: Received 20 November 2006/Accepted 14 February 2007

ABSTRACT

Fecal samples collected from healthy pigs from 13 of the top 17 swine-producing states were tested for *Escherichia coli* O157:H7 as part of the National Animal Health Monitoring System Swine 2000 study. Serogroup O157 strains were isolated from 106 of 2,526 fecal samples. None of the isolates were positive by PCR for the *fliC*<sub>H7</sub> (H7 flagellin) gene or for the *hlyG33* (hemolysin) gene; however, one isolate was positive for the *stx<sub>1</sub>* gene (Shiga toxin 1), an additional four isolates were positive for the *stx<sub>2</sub>* gene (Shiga toxin 2), and three isolates possessed the *eae* gene ( intimin).

*Escherichia coli* is part of the normal intestinal flora of healthy mammals; however, some pathogroups can cause illness in humans and animals. Serological classification of *E. coli* strains depends on the somatic lipopolysaccharide O antigen, the flagellar H antigen, and often also on the capsular K and fimbrial F antigens. *E. coli* strains that cause gastrointestinal illness are characterized based on their pathogenic mechanisms and the presence of specific virulence genes (18). While there are a number of serogroups designated as enterohemorrhagic *E. coli* (EHEC), including *E. coli* serogroups O26, O111, and O103, the major EHEC serotype in the United States is *E. coli* O157:H7. Important EHEC virulence genes include those that encode for the Shiga toxins (*stx<sub>1</sub>* and/or *stx<sub>2</sub>* and the variants), the intimin surface protein (*eae*), and a hemolysin (*hlyG33*) (18).

*E. coli* O157:H7 is an important foodborne pathogen and the predominant cause of hemorrhagic colitis and hemolytic uremic syndrome, in particular in children and the elderly. Cattle are an important reservoir of *E. coli* O157: H7, and human illness has been associated with undercooked ground beef, raw milk, and roast beef, as well as with other foods such as fruits and vegetables contaminated with cattle feces, manure, or water contaminated during harvesting or processing (18, 22). Thus, *E. coli* O157:H7 has had a considerable impact on public health due to its presence in foods that may not be processed and/or cooked properly and also on the food industry due to federal regulations related to food safety.

*E. coli* O157:H7 has been isolated from asymptomatic humans, sheep, turkeys, goats, deer, cats, dogs, rabbits, and birds (3, 7, 10, 16, 18, 19). The pathogen has also been isolated from fecal samples taken from intact pig colons in the United States at a rate of 2.0% (6 of 305) (4). Moreover, *E. coli* O157:H7 isolates have been recovered from the feces of 1.4% (3 of 220) of swine tested in Japan (13) and 0.1% (2 of 1976) of swine tested in Norway (11). In 2000 and 2001, the U.S. Department of Agriculture’s (USDA’s) National Animal Health Monitoring System (NAHMS) conducted a broad study of swine operations in 17 leading production states, representing 94% of the swine production in the United States. In collaboration with the USDA–Agricultural Research Service (ARS), one objective of NAHMS was to describe the prevalence of foodborne pathogens in finisher pigs and identify potential control factors to reduce public health risks due to these pathogens. Therefore, the aim of the current study was to determine the prevalence of *E. coli* O157:H7 in swine feces collected in 2000 and 2001 through the NAHMS survey.

MATERIALS AND METHODS

Study design and sample collection. One aim of the NAHMS study was to provide the pork industry with information regarding foodborne pathogens associated with swine. Selection
of the states to be included in the study was completed in February 1999, based on information in the National Agricultural Statistics Service (NASS) 1 December 1998 Quarterly Hogs and Pigs report. The 17 states selected accounted for 93.6% (ca. 56 million head) of pigs in the United States and 76.4% (65,500) of the U.S. swine operations as of 1 December 2000. Swine operations included in the study had a total of 100 or more pigs as of 1 March 2000. A total of 2,499 randomly selected producers were visited by the NASS personnel, and 895 of these producers were subsequently interviewed. A subset of these producers voluntarily participated and allowed for collection of fecal samples.

A report published by the USDA's Animal and Plant Health Inspection Service (APHIS) and Veterinary Services provides a complete description of the NAHMS 2000 study design (21). Briefly, USDA-APHIS veterinary medical officers collected 2,526 fecal samples from 57 swine farms in 13 of the 17 major swine-producing states (Arkansas, Colorado, Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Nebraska, North Carolina, Ohio, South Dakota, and Wisconsin) between August 2000 and April 2001. Samples consisted of fresh swine feces from late finishers (20 weeks of age or older) and available cull sows (within 10 days of slaughter). Approximately 50 fresh fecal samples were collected from the floor of pens of late finisher pigs, and up to 10 samples were collected from cull sows, when available. The fecal samples were then processed, aliquoted into Whirl-Pak bags (Nasco, Modesto, Calif.), and shipped on ice via overnight carrier in dry ice until receipt.

Fecal samples from a number of different farms. The remaining portion of 1,438 of the fecal samples was then subjected to enrichment on the USDA-ARS Eastern Regional Research Center (ERRC), where testing for E. coli O157:H7, as well as for Shiga toxin-producing E. coli (S. Typhimurium, S. Enteritidis, and S. Typhi), was performed. Therefore, 1,438 and 1,088 fecal samples were tested for the presence of E. coli O157:H7 at the ERRC and RRRC laboratories, respectively, for a total of 2,526 samples. Thus, the samples shipped to the RRRC were tested within 24 h of collection, and those that were then shipped to the ERRC were analyzed within 48 h of collection.

Selective enrichment and bacterial isolation by immunomagnetic separation. Fecal samples were processed as previously described (4). Briefly, 10 g of swine feces was subjected to enrichment for 6 h in 100 ml of brilliant green bile broth (Difco, Becton Dickinson, Sparks, Md.) prewarmed to 37°C. After enrichment, 1-ml aliquots were removed and added to a suspension of E. coli O157 antibody-coated immunomagnetic beads (Dynabeads anti–E. coli O157, Dynal, Oslo, Norway). The resulting suspensions were processed by immunomagnetic separation according to the manufacturer's instructions, with some modifications. Briefly, the bead-bacteria suspensions were incubated at room temperature for 30 min, with continuous mixing on a Belco roller drum (model 7736-10164, Belco Glass, Inc., Vineland, N.J.) at 6 rpm. The bead-bacteria complexes were washed with phosphate-buffered saline (PBS, pH 7.4) containing 0.05% Tween 20, and then were resuspended in 100 µl of PBS-Tween. Fifty microliters of the resuspended bead-bacteria complexes was then plated onto sorbitol MacConkey agar (SMAC; Difco, Becton Dickinson) and onto cefixime tellurite (CT; SMAC Media Cefixime-Tellurite Supplement, Dynal)–SMAC agar, which were then incubated overnight at 37°C.

Phenotypic analysis and serotyping of isolates. From samples containing sorbitol-negative colonies, up to 10 nonsorbitol-fermenting colonies each from SMAC and CT-SMAC agars, were tested for the presence of β-glucuronidase activity and the ability to ferment lactose using E. coli medium containing 4-methylumbelliferyl-β-d-glucuronide (MUG) (EC medium with MUG; Difco, Becton Dickinson) and MacConkey agars (Difco, Becton Dickinson), respectively. Lactose-positive and MUG-negative colonies were tested for O157 latex agglutination (RIM E. coli O157: H7 Latex Test, Remel, Lenexa, Kans.). The O157 latex agglutination-positive isolates were tested for the presence of the H7 antigen with latex agglutination. Manufacturer's instructions were followed for testing for both the O157 and H7 antigens, using the RIM E. coli O157:H7 Latex Test kit.

Genotypic analyses of the isolates by PCR. The latex agglutination-positive isolates were confirmed as serogroup O157 by PCR using primers targeting rfbEO157, a gene encoding perosamine synthetase found in the E. coli O157 O-antigen gene cluster (20). Colonies were mixed with 50 µl of sterile water and heated in a boiling water bath for 10 min. PCR was performed in a GeneAmp 9600 PCR system (Applied Biosystems, Foster City, Calif.). Primers and PCR conditions used for amplification of rfbEO157 were previously described (15). Isolates positive for rfbEO157 were also tested for the presence of genes encoding for the H7 flagellin protein (flhCD), Shiga toxin 1 (stx1), Shiga toxin 2 (stx2), the intimin protein (eae), and a hemolysin (hlh933) by PCR as previously described with minor modifications (4, 6). Briefly, multiplex PCR assays were used to test for the presence of the stx1, stx2, and flhC-H7 genes, using an annealing temperature of 57°C, while the presence of the eae and hlh933 genes were tested using singleplex assays with annealing temperatures of 57 and 54°C, respectively.

Sensitivity of selective enrichment and bacterial isolation. The sensitivity of the method for the isolation of E. coli O157: H7 from swine feces was determined by adding 1 ml of 10-fold dilutions (ranging from 10⁰ to 10⁷ CFU/ml) of an overnight culture of E. coli O157:H7 strain 933 (meat isolate, USDA–Food Safety and Inspection Service) to 10-g samples of fresh feces collected from pig colons obtained from a local swine processing plant. The inoculated feces were added to 100 ml of brilliant green bile broth prewarmed to 37°C. Bacterial isolation and identification were performed as described above. The assay was performed in duplicate and repeated three times.

RESULTS AND DISCUSSION

The sensitivity of the enrichment/detection method was 10 CFU/10 g of swine feces when immunomagnetic beads were plated onto SMAC agar; however, when immunomagnetic beads were plated onto CT-SMAC agar, the detection limit was 1,000 CFU/10 g of swine feces. The differences in sensitivity may be attributed in part to the presence of cefixime and tellurite in CT-SMAC and the adverse effect on growth of E. coli O157 strains in the presence of these selective agents (12, 14).

Swine fecal samples (2,526) collected from 57 farms were examined for the presence of E. coli O157:H7. Ninety-seven percent of the samples (2,454 of 2,526) were obtained from the floors where finisher pigs were housed, and 3% (72 of 2,526) were from cull sows. One hundred six (4.2%) of the 2,526 swine fecal samples analyzed contained isolates that were positive for PCR for rfbEO157, a gene in the O157 O-antigen gene cluster. These strains were isolated from fecal samples from a number of different farms.
Of the 106 isolates that were O157 positive, none was positive by PCR for the fliC_{h7} or for the hly_{933} gene; however, one isolate was positive for the stx_{1} gene, four for the stx_{2} gene, and three for the eae gene. Each of these strains was positive for only one of the virulence genes, and each strain was isolated from different samples collected from various farms.

In comparison to the NAHMS Swine 1995 study, the NAHMS Swine 1995 study tested 4,229 swine fecal samples collected from 152 pork producers in the top 16 swine-producing states (1). In the 1995 study, 25 E. coli O157:NM (nonmotile) strains from 12 farms and 3 E. coli O157: H^{-} (H nontypeable) strains from 2 farms were isolated from the fecal samples. Twenty-nine strains had a rough O phenotype. One rough O strain was positive for the stx_{2} gene, six O157:NM strains from two farms were positive for the eae gene, and one O157 nonmotile strain harbored both stx_{1} and stx_{2} genes. Schroeder et al. (17) detected the presence of stx_{1} and stx_{2} genes in E. coli O157 isolates recovered from swine during 1985 to 2000; however, none of the 17 Shiga toxin–producing isolates was E. coli O157:H7. In another study, Kaufmann et al. (8) examined fecal samples from 630 slaughtered finisher pigs for the presence of E. coli O157 and Shiga toxin–producing E. coli (STEC). They found that 7.5 and 22% were positive for the rfbE and stx genes, respectively. Analysis of 31 E. coli O157 strains showed that 30 of the strains were sorbitol positive and belonged to H types other than H7, while one sorbitol-negative isolate belonged to serotype E. coli O157:H7 and possessed the eae, ehxA (enterohemolysin), and paa (porcine A/E–associated protein) genes. None of the E. coli O157 isolates possessed stx genes. In the current study, no sorbitol-positive E. coli O157 strains were analyzed since only sorbitol-negative isolates were picked from SMAC and CT-SMAC agars. Collectively, these data indicate that E. coli serogroup O157 strains are found in swine; however, Shiga toxin–producing E. coli O157:H7 possessing stx genes, in addition to other EHEC virulence genes, are not commonly shed by healthy pigs.

In the present study, EHEC O157:H7 was not isolated from swine fecal samples collected from pen floors that were subsequently transported on ice and then processed within either 24 or 48 h. In a related study that was also conducted in 2001, we detected E. coli O157:H7 in 6 of 305 (2%) fecal samples that were obtained directly from intact swine colons, transported on ice, and processed within 2 h of collection (4). All of the presumptive E. coli O157:H7 isolates possessed the rfbE_{O157} and fliC_{h7} genes by PCR. Four of six E. coli O157:H7 isolates contained the stx_{1}, eae, and hly_{933} genes, and two contained the stx_{2}, eae, and hly_{933} genes. Despite the use of the same enrichment and isolation procedure that was used to recover the pathogen directly from feces obtained from intact colons (4), E. coli O157:H7 was not isolated from fecal samples obtained in the current study (NAHMS Swine 2000); however, several E. coli O157:non-H7 strains possessed the eae (three isolates), stx_{1} (one isolate), or stx_{2} (four isolates) genes. Keen et al. (9) examined the prevalence of E. coli O157:H7 in livestock, including cattle, swine, sheep, and goats on display at 32 agricultural fairs in the United States. E. coli O157:H7 was isolated from livestock at 31 of the 32 fairs and was found in 1.2% (13 of 1,102) of the swine fecal samples. Zero, six, and seven swine isolates possessed stx_{1}, stx_{2}, or both stx_{1} and stx_{2}, respectively, and all but one isolate possessed eae. Interestingly, nine E. coli O157:H7 swine isolates were negative for stx_{1} and stx_{2}. Indistinguishable pulsed-field gel electrophoresis patterns were observed for isolates recovered from cattle, swine, and flies at one fair, suggesting that flies may be transmission vectors for E. coli O157:H7, or that flies and animals acquired the pathogen from a common source, such as feed or water. The prevalence of Shiga toxin–producing E. coli O157:H7 in swine feces examined by Keen et al. (9) and Feder et al. (4) were similar at 1.2 and 2%, respectively. Keen and co-workers, however, showed that Shiga toxin–negative E. coli O157:H7 strains were also found in swine.

None of the swine fecal samples tested in the current study was positive for E. coli O157:H7. One possibility that could explain why E. coli O157:H7 was not isolated is that the samples were not tested immediately after collection. More specifically, roughly half of the fecal samples were tested within 24 h at the RRRC, and the other half at the ERRC within 48 h of collection. Feder et al. (4) processed swine colon samples within 2 h of collection, and E. coli O157:H7 was isolated from ca. 2% (6 of 305) of the samples tested. The time that elapsed between fecal sample collection and testing was not stated in the study by Keen et al. (9). E. coli O157:H7 may be injured or inhibited by the components in swine feces and/or exposure to cold temperatures during shipment to the laboratory. Enrichment in brilliant green bile broth, a selective medium, may not have allowed for recovery of any injured E. coli O157:H7 cells in the present study. This hypothesis is supported by Weghofer (23), who reported that clinical specimens that were improperly collected and transported and not tested immediately after collection yielded false-negative results.

In a separate study conducted simultaneously with the current study, Fratamico et al. (5) examined a portion (687 samples) of the same fecal samples from the NAHMS 2000 study for the presence of STEC (E. coli O157:H7 and non-O157 STEC). Enrichment was performed in nonselective tryptic soy broth rather than brilliant green bile broth. Although 70% (484 of 687) of the samples were positive for either stx_{1} or stx_{2} or for both toxin genes by PCR, viable STEC were isolated from only 40% of the stx_{1}– and/or stx_{2}–PCR–positive samples. The 219 STEC strains isolated belonged to various STEC serogroups; however, none of the isolates belonged to serotype O157:H7.

In summary, E. coli serogroup O157 isolates were recovered from 106 of 2,526 swine fecal samples obtained from 57 swine farms in 13 of the 17 leading swine-producing states. It was not possible to recover serotype O157:H7 strains of E. coli from any of the 2,526 fecal samples tested. However, EHEC virulence genes (stx_{1}, stx_{2}, or eae) were present in eight O157 isolates that did not possess the fliC_{h7} gene. Additional studies are needed to (1) elucidate why it was possible to detect E. coli O157:H7 in fecal samples obtained directly from pigs (colon or rectal) but not
from samples of pig feces taken from pen floors, (ii) examine the survival of the organism during transport of samples to the laboratory, and (iii) develop enrichment media/isolation methods that allow for recovery of E. coli O157:H7 that may have been stressed or injured in fecal samples during transport.

ACKNOWLEDGMENTS

We thank Mr. Richard Perrine, Ms. Peggy Williamson, Mr. Jeffrey Call, and Ms. Nellie Osoria at the USDA-ARS-ERRC, and Mr. Caleb Lilley and Ms. Jennifer Garner at the USDA-ARS-RRRC for their technical assistance.

REFERENCES