RESEARCH REPORT
PUBLIC HEALTH/WORKER SAFETY

Title: *E. coli* O157:H7 prevalence and characterization from NAHMS 2006 swine fecal samples, **NPB #06-170**

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Industry Summary:

The objectives of the current study were to use the 2006 swine NAHMS samples to determine the prevalence, antimicrobial characterization, and genetic profile of *E. coli* O157:H7 in the U.S. swine herd and to determine the prevalence of *Clostridium difficile* in theU.S. swine herd. Fecal samples from swine farms from across the U.S. were sampled for *E. coli* O157:H7 using the same cultural methods that FSIS uses and for *Clostridium difficile* using two cultural procedures. A total of 1905 samples from 134 farms were sampled for *E. coli* O157:H7. Seven samples from one farm tested positive for *E. coli* O157:H7, but all were found not to have the toxin gene which would make them avirulent. A total of 864 samples from 60 farms were tested for *C. difficile*. Using the best available method at the time the study was started (single alcohol shock), 5.44% of the samples were found to be positive for *C. difficile*. However, about half way through the study a more sensitive method (double alcohol shock) was discovered and using this method 15.65% of 345 samples were shown to be *C. difficile* positive. Ribotyping suggests that no strains of *C. difficile* from this study are the same as the hypervirulent strain which has been causing problems in human patients in Canada and Europe. Further genetic characterization studies are continuing. The results from this study suggest that little to no *E. coli* O157:H7 is seen in fecal samples from U.S. swine. These results confirm the results from the 1996 NAHMS swine study where no *E. coli* O157:H7 were found. Further, *C. difficile* is found in about 15% of the swine fecal samples, but the strains present do not appear to be the hypervirulent strain that has recently caused so many hospital patient or community acquired problems.

Abstract

E. coli O157:H7 has been recognized as a foodborne pathogen since 1982.It is estimated that there are more than 73,000 symptomatic infections annually in the U.S. from food exposure, resulting in more than 1,800 hospitalizations and 61 deaths (Mead *et al.*, 1999). There is particular concern for children under the age of five where a disproportional incidence rate occurs and as many as 3000 cases of hemolytic uremic syndrome may occur annually.

Epidemiology studies suggest that ground beef is the primary foodborne source of human exposure to *E. coli* O157:H7. Early studies in Germany (Gallien *et al.*, 1994), Australia (Sidjabat-Tambunan and Bensink, 1997), and Canada (DesRosier *et al.*, 2001) did not find any *E. coli* O157:H7 in swine. These studies were corroborated in the 1996 U.S. swine NAHMS study (Bush, 1997) where no *E. coli* O157:H7 was seen in 4,229

samples from 152 herds in 6 states. However, others have reported low levels of *E. coli* O157:H7 in Japan (Nakazawa and Akiba, 1999), Norway (Johnsen *et al.*, 1999), Chile (Rios *et al.*, 1999). Improvements in sensitivity of methods to recover *E. coli* O157:H7 including inclusion of immuno-magnetic beads to concentrate these pathogens and rainbow agar to better distinguish colonies have been widely recognized and led to a revision of the USDA, FSIS microbiological laboratory guidebook in 2002 (Weagant *et al.*, 1995, Taormina *et al.*, 1998). These changes led to a substantial increase in recovery of *E. coli* O157:H7 from ground beef and other products.

After using more sensitive methods for recovery, Feder and co-workers (2003) found *E. coli* O157:H7 in about 2% of 305 colon fecal samples in the U.S. All isolates recovered in this study were sensitive to the antimicrobial agents tested with the exception to one isolate that was resistant to streptomycin. In this study, there were no difference in ribotype patterns of the isolates, but there were four distinct PFGE patterns. The objective of the current study was to determine the prevalence, antimicrobial characterization, and genetic profile of *E. coli* O157:H7 from U.S. swine.

Clostridium difficile is a spore-forming bacterium that has been associated with severe colitis. Historically thought of as a hospital acquired enteric disease primarily in patients with severe underlying medical problems, recently published information suggest that *Clostridium dificile* may be associated with foods of animal origin (Rodriguez-Palacios *et al.*, 2007). The epidemiology of *C. difficile*-associated diarrhea (CDAD) appears to have changed with increased illness and relapse rates (Pepi *et al.*, 2005, Pituch *et al.*, 2006) associated with the emergence of one toxigenic strain, classified according to PCR as ribotype 027/toxinotype III and pulsed-field gel electrophoresis (PFGE) as NAP1 (Warny, *et al.*, 2005) *C. difficile* which are indistinguishable from human isolates have also been associated with enteric diseases in animals including pigs (Arroyo, *et al.*, 2005). The potential for these non-hospital or community acquired strains of *C. difficile* to be associated with human illnesses have increased the need to better assess the prevalence and genetic types of *C. difficile* from agricultural animals including pigs.

Objectives

This study was the first in the U.S. using more sensitive methods for isolation and identification of *E. coli* O157:H7 currently now used by the regulatory agencies. The data permits the swine industry to know more precisely if there are any potential issues with *E. coli* O157:H7 and if so are there any antimicrobial resistance issues which would be unique to the swine industry. The second objective of this study was to determine the prevalence and genetic type of *C. difficile* associated with U.S. swine.

Materials & Methods

As part of the 2006 Swine NAHMS study, a total of 1905 samples from 134 farms were sampled for *E. coli* O157:H7. All confirmed *E. coli* O157:H7 isolates will be analyzed to determine antimicrobial resistance profiles. Between September, 2006 and February, 2007 employees or contractors of USDA, APHIS will collect fecal samples from selected farms in 17 states. Sixty samples from each farm will be shipped overnight on ice to the USDA, ARS in Athens, Georgia.

***E. coli* Methods**

Approximately every fourth sample (15/farm) will be sampled for *E. coli* O157:H7 using standard cultural methods with immuno-magnetic bead concentration. For all confirmed *E. coli* O157:H7 isolates, antimicrobial resistance profiles was determined using custom made plates containing up to 16 antimicrobics in a semi-automated minimal inhibitory concentration format system (Sensititer^{TM}, Trek Diagnostic) at the USDA, ARS in Athens, GA. Isolates were classified as susceptible, intermediate, or resistant based on Clinical Laboratory Standards Institute (CLSI) (formerly National Committee for Clinical Laboratory Standards) established breakpoints, where available. Additional genotyping will include Pulse Field Gel Electrophoresis (PFGE) analysis to assess relatedness. Additional genotyping of isolates and use of microarrays to assess gene and virulence attributes will be conducted where possible.

***Clostridium difficile* Cultural Methods**

A total of 864 samples from 60 farms were tested for *C. difficile*.

Single alcohol shock (Arroyo *et al*., 2005): One fecal swab was placed into 9.0 ml of cycloserine-cefoxitin fructose broth supplemented with 0.1% sodium taurocholate (TCCFB). After 7 days of incubation at 37°C, 2.0 ml was transferred into a sterile test tube and mixed with an equal amount of absolute ethanol and left at room temperature for 60 min. After leaving at room temp for 60 min, the samples were centrifuged at 3,800x g for 10 min. The resulting pellet was plated onto blood agar (BA) and *Clostridium difficile* cycloserine-cefoxitin fructose agar (CCFA). Incubate anaerobically at 37°C for 48 h. Subculture suspicious colonies then identify by distinct odor, fluorescent under UV light, colony morphology, Gram stain appearance, and production of L-proline aminopeptidase (Pro-Disc; Remel, Carr-Scarborough Microbiologicals, Inc., Decatur, GA). All positive isolates were kept in cooked meat medium.

Double alcohol shock (Arroyo *et al*., 2006): One g or 1 ml of feces was mixed with an equal volume of absolute ethanol, vortexed, and left at room temp for 60 min. The samples were then centrifuged at 3,800x g for 10 min. The resulting pellet was placed into 9.0 ml TCCFB and incubated anaerobically at 37°C for 7 days. After incubation, 2.0 ml was transferred into a sterile test tube and mixed with an equal amount of absolute ethanol and left at room temperature for 60 min. After leaving at room temp for 60 min, the samples were centrifuged at 3,800x g for 10 min. The resulting pellet was plated onto BA and CCFA. Subculture suspicious colonies then identify as the same criterions as the single alcohol shock method.

Results

A total of 1905 samples from 134 farms were sampled for *E. coli* O157:H7. Seven samples from one farm tested positive for *E. coli* O157:H7, but all were found not to have the toxin gene; this renders them avirulent.

A total of 864 samples from 60 farms were tested for *C. difficile*. Using the best available method at the time the study was started (single alcohol shock), 5.44% of the samples were found to be positive for *C. difficile*. However, about half way through the study a more sensitive method (double alcohol shock) was discovered and using this method 15.65% of 345 samples were shown to be *C. difficile* positive. Ribotyping suggests that no strains of *C. difficile* from this study are the same as the hypervirulent strain which has been causing problems in human patients in Canada and Europe. Further genetic characterization studies are continuing.

Discussion

The results from this study suggest that little to no *E. coli* O157:H7 is seen in fecal samples from U.S. swine. These results confirm the results from the 1996 NAHMS swine study where no *E. coli* O157:H7 were found. Further, *C. difficile* is found in about 15% of the swine fecal samples, but the strains present do not appear to be the hypervirulent strain that has recently caused so many hospital patient or community acquired problems.

References:

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