Survey of *Clostridium difficile* in Food Animals and Retail Meats

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Abstract

Several small surveys and one large survey were conducted in Texas in order to determine the prevalence of *Clostridium difficile* (Cd) in food producing animals and retail meats. Composite fecal samples (n = 1008) from various age groups of commercial swine and pork trim (n = 62) from a sausage processing plant were collected in 2006 from an integrated swine operation. Samples were collected from commercial poultry and consisted of feces from 70-week-old laying hens (n = 50) and cecal contents from 28-day-old broiler chickens (n = 36). Cattle fecal samples were collected weekly from 14, 1-year-old dairy calves (n = 64). We collected 40 retail meat samples from 5 different stores on 4 separate dates, and samples consisted of ground pork, ground beef, ground chicken, ground turkey, pork sausage, pork and beef summer sausage, pork longaniza, pork chorizo, and pork beer bratwurst. Isolation techniques utilized enrichment, concentration, and restrictive media. We isolated Cd from 131/1008 (13.2%) swine feces, 4/62 (6.5%) pork trim, 0/86 (0%) poultry sources, 1/64 cattle feces (1.6%), and 3/40 (7.5%) retail meats (1 pork chorizo, 1 ground turkey, 1 ground pork). The prevalence of Cd from swine was more pronounced in suckling piglets (36.5%) compared to older animals (3.4%). All of the meat and trim isolates, and the majority of swine isolates, were toxigenotype V. Sensitivities to 11 antibiotics were similar for Cd from animals and meat in our study. We conclude that Cd has a low prevalence in market-age animals and in retail meats.

Introduction

Since 2003, the incidence and severity of disease associated with toxigenic *Clostridium difficile* (Cd) have increased in hospitals in North America (1, 2). These increases have coincided with the emergence of a new epidemic strain (BI/NAP1, toxigenotype III) that has increased virulence and resistance. The origins of this strain have yet to be determined. Various strains of Cd, including NAP1, can be isolated from food animals and retail meat (3-5); however, the predominant strain from food animals is NAP7 (3). Because Cd has been isolated from food animals and meat, it has been proposed that Cd may be transmitted via food sources (3-5, 6). The objectives of the present study are to: 1) compare the Cd prevalence in different production groups of swine and pork products in an integrated swine operation in Texas, 2) determine the prevalence of Cd in various food animals, and 3) examine the occurrence of Cd in retail meat.

Materials and Methods

*Sample Collection:* Composite fecal samples (n = 1008, representing approximately 10 pigs/sample) were collected February 2006 to January 2007 from asymptomatic, clinically healthy multiple-age swine from 13 separate farms at 3 geographical locations in Texas. Pork trim samples (n = 62) were collected from a sausage processing plant from the same operation. In 2007, fecal samples were collected from 70-week-old Leghorn laying hens (n = 50) and cecal samples from 28-day-old broiler chickens were collected from commercial sources. Weekly fecal samples (n = 64) were collected in 2009 from 14, 1-year-old dairy calves. On 4 separate dates during 2008-2009, we collected 40 retail meat samples from 5 different meat markets and grocery shelves from stores in Bryan and College Station, TX. Samples collected were ground pork, ground beef, ground chicken, ground turkey, pork sausage, pork and beef summer sausage, pork longaniza, pork chorizo, and pork beer bratwurst and were a mix of store brands and national brands.
Cultivation of Cd: Cultivation of Cd was accomplished by alcohol shock, anaerobic incubation, enrichment/concentration techniques, and use of restrictive media. Cultivation of feces followed the procedures as described by Rodriguez-Palacios et al., 2006 (3), whereas the techniques for meat cultivation consisted of increased sample size and enrichment time (5).

PCR for toxin A&B genes, regulatory gene deletion, toxingotyping, and binary toxin gene:
DNA extraction was accomplished by the QIAamp DNA mini-kit (QIAGEN Sciences, Germantown, MD). PCR procedures for tcdA, tcdB, cd/B binary toxin, toxingotyping, and tcdC gene detection followed the techniques according to PCR protocols as utilized by the Centers for Disease Control and Prevention (CDC), Atlanta, GA (7-9).

Antibiotic resistance:
All of the swine-study and meat isolates were tested for susceptibility to eleven antibiotics (ampicillin, chloramphenicol, tetracycline, amoxicillin/clavulanic acid, imipenem, cefoxitin, metronidazole, ciprofloxacin, clindamycin, piperacillin/tazobactam, and vancomycin) by use of the Etest (AB Biodisk North America, Inc., Piscataway, NJ) according to the manufacturer's recommendations.
Results for minimum inhibitory concentrations (MIC) were interpreted according to standard criteria except MIC's for ciprofloxacin were based on values for trovafloxacin, whereas vancomycin interpretation was based on MIC's reported for Gram positive aerobes. Quality control strains were tested using recommended breakpoints for MIC's (10).

Statistical analyses:
Statistical analyses (in XTLOGIT procedure:Stata SE Release 10.1, Stata Corp., College Station, TX) were those appropriate for time-series cross-sectional data (11).

Results and Discussion
Of the 1008 swine samples tested, 131 were positive for Cd (13.2%). The highest prevalence of Cd was observed in the farrowing barn (36.5%), followed by nursery pigs (8.2%), pork trim (6.5%), grower/finisher pigs (3.9%), and breeding swine (3.8%), respectively (Table 1). Our results are in agreement with other reports in that young animals such as piglets and calves appear to have increased carriage compared to more mature animals (3, 4, 6). In the present study, there were no differences (P>0.05) in Cd isolations when compared by season. However, when prevalence data were collapsed into fall-winter (Sept.-Feb.) or spring-summer (Mar.-Aug.), there were significantly (P<0.001) more isolations during the cooler months. PCR testing for toxin genes in the swine isolates determined that 122 were positive for toxin A and B genes (tcdA, tcdB), 129 had 39 bp deletion in the tcdC regulatory gene, 131 were positive for binary toxin gene (cd/B), and 120 were toxingotype V. Our results agree well with those of other animal studies (3, 4, 6, 11). All (100%) were resistant to cefoxitin, ciprofloxacin, imipenem, 92% resistant to clindamycin, 53% intermediate to ampicillin, and 100% sensitive to metronidazole, vancomycin, piperacillin/tazobactam, and amoxicillin/clavulanic acid, whereas 90% and 98% were sensitive to tetracycline and chloramphenicol, respectively. These results are somewhat similar, although with slightly less cumulative resistance, to antibiotic sensitivities for animal isolates (3), but are considerably less resistant compared to human clinical isolates (1). Four Cd were isolated from 62 pork trim samples and were determined to be toxingotype V, NAP7 and had antibiotic sensitivities that were similar to the swine isolates.
None of the laying hen or broiler samples were positive for Cd, whereas 1/64 of dairy calf samples were positive. There were no meat samples positive when using the same enrichment procedure for swine, calf, and chicken samples, but 3/40 were positive when an extended enrichment time was used. The isolates came from ground pork, pork chorizo, and ground turkey, all from the same store. The pork chorizo and ground turkey were national brands, whereas the ground pork was a store brand. The isolates were toxingotype V and similar to PFGE type-NAP 7 isolates from cattle and swine (11). The antibiotic sensitivities were similar to those of the pork trim and swine isolates.
On the basis of the results of our swine study, we conclude that young animals have a higher prevalence of Cd than older swine cohorts. Although the strain of Cd from our swine isolates (toxingotype V, PFGE NAP 7) has been recovered from humans, none of our swine isolates were of the human epidemic strain (toxingotype III, PFGE NAP1). If Cd is indeed a food-associated organism, we speculate that the lower carriage of Cd in market-age animals in our study would suggest potentially reduced transmission risk to
the food chain. From our limited studies, we propose that poultry, cattle, and retail meats have a very low prevalence of Cd. On the basis of our experience, we conclude that cultivation techniques for Cd are highly variable, there are no validated procedures for animal feces and meat, and we question the sensitivities of existing techniques for Cd recovery from environmental sources.

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References
11. STATA STATISTICAL SOFTWARE: SE release 10.1 Longitudinal/Panel Data [XT], 2007. Stata Corp LP, College Station, TX, pp. 205-21.

Table 1. Clostridium difficile prevalence (number positive/number tested) by production group in an integrated swine operation.

<table>
<thead>
<tr>
<th>Production Group</th>
<th>Positive/Tested (%)</th>
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<tr>
<td>Farrow</td>
<td>95/260 (36.5)</td>
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<tr>
<td>Nursery</td>
<td>10/122 (8.2)</td>
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<tr>
<td>Pork Trim</td>
<td>4/62 (6.5)</td>
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<tr>
<td>Grow/Finish</td>
<td>15/382 (3.9)</td>
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<tr>
<td>Breeding Sows/Boars</td>
<td>7/182 (3.8)</td>
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<tr>
<td><strong>Totals:</strong></td>
<td><strong>131/1008 (13.2)</strong></td>
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