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Prevalence and Genotypic Characteristics of *Clostridium difficile* in a Closed and Integrated Human and Swine Population[▽]

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Recently, an apparent rise in the number of cases attributed to community-acquired *Clostridium difficile* infection has led researchers to explore additional sources of infection. The finding of *C. difficile* in food animals and retail meat has raised concern about potential food-borne and occupational exposures. The objective of this study was to compare *C. difficile* isolated from a closed population of healthy individuals consisting of both humans and swine in order to investigate possible food safety and occupational risks for exposure. Using a multistep enrichment isolation technique, we identified 11.8% of the human wastewater samples and 8.6% of the swine samples that were positive for *C. difficile*. The prevalences of *C. difficile* in swine production groups differed significantly ($P < 0.05$); however, the prevalences in the two human occupational group cohorts did not differ significantly ($P = 0.81$). The majority of the human and swine isolates were similar based on multiple typing methods. The similarity in *C. difficile* prevalence in the human group cohorts suggests a low occupational hazard, while a greatly decreased prevalence of *C. difficile* in later-stage swine production groups suggests a diminished risk for food-borne exposure. The similarity of strains in the two host species suggests the possibility of a common environmental source for healthy individuals in a community setting.

Clostridium difficile has been recognized as one of the leading bacterial causes of nosocomial diarrhea and pseudomembranous colitis in hospitals and nursing homes since the 1970s. The emergence of community-acquired cases has recently led researchers to search for additional sources of infections (11, 18). Patients with no recent history of prior hospitalization are typically classified as community-acquired cases (7, 32). Several studies have shown that a history of antibiotic use is not only a risk factor for nosocomial infection but also for community-acquired infection (18, 36); however, another study found that 35% of community-acquired cases had no history of hospitalization or antibiotic use (48), and there have been published reports of cases with no history of antibiotic use (8). Some of the other possible sources or risk factors for these community-acquired infections under investigation include food-borne exposure, companion and food animal exposure, environmental exposure, and concurrent use of proton pump inhibitors (2, 12, 13, 21, 28, 35, 38, 39, 41, 50).

Clostridium difficile has been isolated from food animals, including swine, chickens, and cattle (21, 35, 40–42, 50). *C. difficile* was first discovered in swine in 1980 when gnotobiotic pigs were accidentally exposed to the bacterium (31) and has since been found to be one of the primary agents responsible for diarrhea in piglets (47, 49). The prevalence of *C. difficile* in piglets has been reported to range from 25.9% (4) to around 50% (6, 33) and even as high as 74% (47). The majority of strains isolated from piglets are toxinotype V, ribotype 078, pulsed-field gel electrophoresis (PFGE) type NAP7 (North American pulsed-field type 7) (15, 25, 35). Some strains isolated from swine have shown as much as 100% similarity to those isolated from humans (21). Retail meats have also proven to be a source for *C. difficile* (39, 42). The finding of *C. difficile* in food animals and retail meat raises concern for the potential for both food-borne and occupational exposures. The objective of this study was to compare the prevalence and genotypic characteristics of *C. difficile* isolated from a closed healthy population consisting of both humans and swine to investigate possible food safety and occupational risks associated with *C. difficile* in swine.

MATERIALS AND METHODS

Sampling. Swine composite fecal samples and human composite wastewater samples were collected from a closed, vertically integrated population in the state of Texas. The population consisted of 12 units in different geographical locations that contained both a human and swine population; in addition, there was also a single on-site slaughter plant facility dedicated to the processing of these pigs. There was little movement into or out of the system by either the swine or human population.

The human population consisted of occupational group cohorts of individuals who work with swine (swine workers) and individuals who do not work with swine (nonswine workers). The two occupational cohorts were housed separately, and the only difference between the two populations was their exposure to swine. All individuals had equal opportunity to consume pork produced within the system. The swine population flowed vertically from the farrowing barn to the grower/finisher slabs; thereafter, all finished swine were slaughtered and consumed

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within the closed agricultural food system. Swine production groups sampled included farrowing, nursery, grower/finisher, and breeding cohorts.

Human and swine composite fecal samples were collected monthly from February 2004 through January 2007. Sampling methods for the swine population have been previously described (33). Composite wastewater grab samples were collected from representative sewage manholes (i.e., directly draining lavatories of the two representative occupational cohorts) into 50-ml tubes. The wastewater systems were closed and not affected by rainwater or surface runoff. The sampling locations were chosen to differentiate between the occupational cohorts, and typically 3 swine worker wastewater samples and 3 nonswine worker wastewater samples were collected from each of the 13 units. Samples were stored on ice and shipped to the Food and Feed Safety Research Unit (FFSRU) laboratory, Agricultural Research Service (ARS), U.S. Department of Agriculture (USDA), College Station, TX. Upon arrival, the wastewater samples were stirred to ensure uniformity, and then 4 ml was placed into a 5-ml tube containing 1 ml of sterile glycerol and stored at −80°C.

Isolation of bacteria. Isolation of *C. difficile* from swine fecal samples was performed utilizing an enrichment procedure, alcohol shock treatment, and restrictive medium technique previously described (33). Isolation of bacteria from the more-diluted human wastewater samples was performed in a similar manner as for the swine samples except for one modification during the plating step. In an anaerobic chamber, 1 g of wastewater sample was added to a 15-ml tube containing 2 ml of 96% ethanol. The samples were aerobically agitated for 50 min and then centrifuged at 3,800 × *g* for 10 min. In an anaerobic chamber, the supernatant was removed from the tubes, and the sediment was suspended in 5 ml of cycloserine-cefoxitin-fructose broth (CCFB) (33). The enriched samples were incubated for 7 days anaerobically at 37°C. On the seventh day, 5 ml of 96% ethanol was added to the tubes anaerobically, and the tubes were centrifuged aerobically at 3,800 × *g* for 10 min. The supernatant was removed anaerobically, the sediment was suspended in 200 μl of sterile deionized water, and 200 μl of the suspended sediment was spread onto a cycloserine-cefoxitin-fructose agar (CCFA) plate (Anaerobe Systems, Walnut, CA). The plates were incubated anaerobically at 37°C and checked daily for growth for 5 days.

Molecular methods. Isolation of the DNA was accomplished by the QIAamp DNA minikit (Qiagen Sciences, Germantown, MD). PCR analysis for the presence of toxin genes, *tcdC* gene deletion, binary toxin gene, and toxinotyping for isolates from both the human and swine wastewater samples were performed using methods previously described (23, 24, 26). Pulsed-field gel electrophoresis (PFGE) was performed using a modified technique utilized by the U.S. Centers for Disease Control and Prevention (26).

Statistical methods. Descriptive statistics, both within and between host species, were generated using cross tabulations by year, month, season, location, and production group/occupational group cohort. Multilevel mixed-effect logistic regression (Stata SE Release 10.1; Stata Corp., College Station, TX), including random intercepts for unit and year in the model to account for the dependence of responses on location and time, was used to explore risk factor associations both within and between host species.

RESULTS

Swine descriptive statistics. A total of 2,936 swine composite samples were tested, and 252 of the samples (8.6%) were culture positive for *C. difficile*. The prevalence of *C. difficile* varied across the 3 years from 8.6% in 2004 to 3.9% in 2005 with a high of 13.6% in 2006. The prevalence was significantly ($P < 0.05$) different among the production groups with the highest prevalence (24.9%) identified in the farrowing barn and the lowest prevalence (2.7%) identified in the grower/finisher swine. The prevalence did not differ significantly ($P = 0.96$) between the seasons. The average monthly prevalence was 8.5% and varied from a high of 12.1% in September to a low of 5.0% in July. Across the 12 swine production units, the prevalence varied from 14.6% to 0.9%. Units one, five, six, and seven had the highest prevalence, and all four of these units were farrow-to-finish units (Table 1).

Human descriptive statistics. There were 2,292 human wastewater samples tested, and 271 of the samples (11.8%) were culture positive for *C. difficile*. The prevalence of *C. difficile* varied across the 3 years from 10.0% in 2004, 18.6% in 2005, and 5.8% in 2006. There was no significant difference ($P = 0.42$) in the prevalence of C. *difficile* between the swine worker and nonswine worker occupational group cohorts. The prevalence of *C. difficile* differed significantly ($P < 0.05$) between the seasons, with a higher prevalence (16.3%) during the spring, which included the months of March, April, and May. The average monthly prevalence was 11.6% and varied from a high of 22.2% in May to a low of 4.9% in February. Across the units, the prevalence varied from a low of 7.6% in unit 12 to a high of 17.2% in unit 7 (Table 1).

Swine molecular results. The majority of the swine isolates ($n = 236$; 93.7%) were toxinotype V. The other toxinotypes found included 7 toxinotype V-like isolates, 7 toxinotype XI isolates, and 2 toxinotype 0 isolates (Table 2). Sixty-six (26.2%) of the isolates were PFGE type NAP7 (North American pulsed-field type 7). The most commonly identified PFGE pattern (173 isolates; 68.7%) was a NAP7 variant pattern that was 90.5% similar to type NAP7 by dendrogram analysis.

Human molecular results. Toxinotyping by PCR revealed 229 (84.5%) of the human wastewater isolates as toxinotype V, 26 (10.7%) as toxinotype 0, 7 (2.6%) as toxinotype V-like, 5 (1.8%) as toxinotype XI, and 4 (1.5%) as toxinotype III (Table 2). The majority of the human isolates were of either the PFGE type NAP7 (23.6%) or the NAP7 variant (66.8%) pattern.

Multilevel mixed-effect logistic regression models within host species. A multilevel mixed-effect logistic regression model for the swine population showed that production group added significantly ($P < 0.001$) to the model, whereas season ($P = 0.83$) and month ($P = 0.31$) were not important. A large component of the variance for *C. difficile* prevalence initially attributed to unit-to-unit differences was instead explained by the two major production group types housed across the units (i.e., farrow-to-finish units versus grower-to-finisher units). In the intercept-only model, 54.4% of the variance was attributed to the unit, whereas in the final model that included production groups only, 32.3% of the variance was attributed to the unit. The multilevel mixed-effect logistic regression model for the human population identified that season ($P < 0.01$) was significant in the model; however, occupational group cohort was not important ($P = 0.93$).

Multilevel mixed-effect logistic regression model across host species. A multilevel mixed-effect model was used to test the association between the fixed factors of host species or swine production group/human occupational group cohort (colinear/nested within host species), season, month, and the interaction of these factors. Either host species ($P = 0.05$) or swine production group/human occupation group cohort ($P < 0.001$) were significant predictors of *C. difficile* prevalence. The season ($P = 0.16$) and month ($P = 0.08$) were not significant predictors. However, when season was forced into the model with either host species or production group/group cohort, we found that both factors became highly significant ($P < 0.001$). The interaction terms of host species and season were also significant ($P < 0.05$); however, the interaction term of production group/group cohort and season were not significant ($P = 0.06$) (Table 3).

TABLE 1. *Clostridium difficile* prevalence in the swine and human populations

General risk factor	Specific risk factor[a]	*C. difficile* prevalence (%) in swine (no. of samples)	95% CI[b] (%) for prevalence in swine	*C. difficile* prevalence (%) in human wastewater (no. of samples)	95% CI (%) for prevalence in human wastewater
Year	2004	8.6 (86)	6.9–10.3	10.0 (82)	8.0–12.1
	2005	3.9 (39)	2.7–5.1	18.6 (150)	15.9–21.3
	2006	13.6 (127)	11.4–15.8	5.8 (39)	4.1–7.6
Production group	Farrowing	24.9 (175)	21.7–28.1		
	Nursery	5.1 (14)	2.5–7.7		
	Breeding	4.3 (26)	2.7–5.9		
	Grower/finisher	2.7 (37)	1.9–3.6		
Occupational group cohort	Swine worker			12.0 (131)	10.1–14.0
	Nonswine worker			11.6 (140)	9.8–13.5
Season/month					
Fall	September	12.1 (49)	7.9–16.3	10.1 (21)	6.0–14.2
	October	7.6 (38)	4.1–11.0	10.6 (21)	6.3–14.9
	November	7.3 (34)	4.1–10.6	8.3 (16)	4.4–12.2
	Fall	9.0 (63)	6.7–11.1	9.7 (58)	7.3–12.1
Winter	December	7.6 (44)	3.9–11.3	15.8 (29)	10.5–21.0
	January	8.9 (46)	5.5–12.4	11.4 (23)	7.0–15.8
	February	9.3 (32)	5.7–12.9	4.9 (9)	1.8–8.0
	Winter	8.7 (61)	6.6–10.8	10.7 (61)	8.1–13.2
Spring	March	8.4 (50)	4.9–11.8	14.9 (29)	9.9–20.0
	April	8.7 (44)	5.4–12.1	11.3 (20)	6.6–16.0
	May	8.3 (67)	5.1–11.5	22.2 (43)	16.3–28.0
	Spring	8.5 (69)	6.6–10.4	16.3 (92)	13.2–19.3
Summer	June	10.7 (41)	7.0–14.3	6.1 (12)	2.8–9.5
	July	5.0 (35)	2.1–7.9	13.5 (24)	8.4–18.5
	August	8.4 (43)	4.8–12.0	13.1 (24)	8.2–18.0
	Summer	8.2 (59)	6.2–10.2	10.8 (60)	8.2–13.4
Unit	1 (F)	14.6 (59)	11.3–18.4	10.6 (17)	6.3–16.4
	2 (G)	6.5 (8)	2.8–12.3	13.0 (21)	8.2–19.1
	3 (F)	1.9 (7)	0.8–4.0	15.7 (26)	10.5–22.1
	4 (G)	2.5 (3)	0.5–7.3	10.8 (23)	7.0–15.8
	5 (F)	10.9 (49)	8.2–14.2	12.8 (20)	8.0–19.1
	6 (F)	14.5 (57)	11.2–18.4	9.1 (17)	5.4–14.2
	7 (F)	11.6 (47)	8.6–15.1	17.2 (28)	11.7–23.9
	8 (G)	4.6 (6)	1.7–9.8	9.9 (21)	6.2–14.7
	9 (G)	1.6 (2)	0.2–5.7	13.7 (19)	8.4–20.5
	10 (G)	0.9 (1)	0.02–4.8	10.1 (20)	6.3–15.2
	11 (G)	4.1 (5)	1.3–9.3	14.1 (29)	9.6–19.6
	12 (G)	4.1 (8)	1.8–8.0	7.6 (13)	4.1–12.6
	13 (S)			10.8 (17)	6.4–16.7
Total		8.6 (252)	7.6–9.6	11.8 (271)	10.5–13.1

[a] The unit type is shown in parentheses after the unit number as follows: F, farrow-to-finish unit; G, grower-finisher; S, slaughter plant.
[b] 95% CI, 95% confidence interval.

TABLE 2. PCR toxinotyping, toxin gene, *tcdC* gene deletion, and binary toxin results for the *Clostridium difficile* isolates from the swine and human populations

Toxinotype	No. of isolates from: Swine	No. of isolates from: Humans	Toxin genes[a]	*tcdC* gene deletion (bp)	Binary toxin
0	2	26	A+ B+	0	Negative
III	0	4	A+ B+	18	Positive
V	236	229	A+ B+	39	Positive
V-like	7	7	A− B+	39	Positive
XI	7	5	A− B−	39	Positive

[a] A+ B+, A positive and B positive; A−, A negative; A− B−, A negative and B negative.

DISCUSSION

The population used to explore the hypotheses in this study is unique because it was closed, with little movement of the two host species in or out of the system, and it contained an integrated set of occupational or production cohorts. Previously published studies regarding potential for transmission of *Clostridium difficile* between food animals and humans have compared isolates arising from completely separate populations (5, 10, 14, 25). This is the first study to explore the potential transmission of *C. difficile* between food animals and humans in

TABLE 3. Coefficients and odds ratios from the multilevel mixed-effect logistic regression model for both host species[a]

Intercept or risk factor	LRT χ^2; *P* value (df)[b]	Category	Coefficient	Adjusted odds ratio	95% confidence interval for the odds ratio
Intercept			−2.57		
Risk factors					
Host species	3.98; 0.05 (1)	Swine (referent)			
		Human	0.39	1.48	1.01–2.17
Season	0.45; 0.93 (3)	Winter (referent)			
		Spring	−0.04	0.96	0.67–1.38
		summer	−0.03	0.97	0.66–1.41
		Fall	0.07	1.07	0.74–1.56
Host species and season interaction	9.14; 0.03 (3)	Swine-winter			
		Human-winter			
		Swine-spring			
		Human-spring	0.56	1.75	1.06–2.91
		Swine-summer			
		Human-summer	0.06	1.06	0.62–1.81
		Swine-fall			
		Human-fall	−0.15	0.86	0.50–1.46

[a] A likelihood ratio test (LRT) of random- vs. fixed-effect logistic regression: $\chi^2_2 = 37.62$; $P < 0.00001$. In the model we used, host species, season, and the interaction of host species and season were treated as the fixed factors, and unit and time were treated as the random effects.

[b] The chi-square values are from an LRT [2(log likelihood in the full model − log likelihood of the reduced model)], used to test the contribution of a subset of parameters to the model.

the same closed population. This is also the first study to assess the occupational risk of *C. difficile* infection from food animals, specifically, from human exposure to swine.

The prevalence of *C. difficile* among the swine production groups was compared in order to determine the potential risk of human infection due to food-borne exposure. *C. difficile* is known to cause diarrhea and pseudomembranous colitis in piglets (43); however, little is known about the presence of this bacterium in the other swine production groups. Consistent with other published studies, the highest prevalence of *C. difficile* was identified in samples from the farrowing barn (3, 49). A much lower prevalence of *C. difficile* was isolated from the grower/finisher pigs (Table 1), and this may be indicative of a lowered food-exposure risk in slaughter-ready pigs. These data are supported by two recent studies that explored the prevalence of *C. difficile* in late-stage production groups and did not isolate the bacteria from finishing pigs (45) or pigs at slaughter (19). The decreasing prevalence of *C. difficile* with the age of the pig has also been reported in another study that found prevalence significantly declined with piglet age in piglets sampled on days 2, 7, 30, 44, and 62 (47). At the farm level, the highest prevalence of *C. difficile* in swine was identified in the farrow-to-finish units in comparison with the grower-finisher units (Table 1). Using multilevel mixed-effect logistic regression models, we identified that this can mostly be explained by the high prevalence of *C. difficile* among the piglets in the farrowing barn.

An overall *C. difficile* prevalence of 11.8% was estimated among the human composite wastewater samples. There has been no other previously published data regarding *C. difficile* in human wastewater. The prevalence in the human wastewater samples was higher than expected; 3% of healthy adults are estimated to be carriers of C. *difficile* (30), and our samples were derived from primarily asymptomatic individuals and heavily diluted with domestic potable water. However, since the samples were aggregated rather than individual samples and our technique included enrichment steps, this may help to explain the higher prevalence. It is important to note that since we used aggregate samples, the estimates of prevalence are not representative of individual prevalence values for either humans or swine. Another potential reason the prevalence in the human wastewater samples is higher than expected is that the dynamics of bacterial growth within wastewater is unknown. Wastewater samples may contain components that enhance or hinder the survival of *C. difficile*. *C. difficile* has been isolated from chlorinated tap water at a very low prevalence and from untreated water in lakes and streams at a much higher prevalence (2). The prevalence of *C. difficile* found in the wastewater samples in this study may reflect the background level of *C. difficile* found in untreated sewage.

The risk of acquiring *C. difficile* from occupational exposure to swine was assessed by comparing the prevalence of *C. difficile* in human wastewater samples arising from each of the swine worker and nonswine worker group cohorts. No significant difference in prevalence was identified between the occupational group cohorts. Although a background level of *C. difficile* may exist in the wastewater samples, this level would be equivalent across the occupational groups. The only difference between the two populations is their occupational exposure to swine; therefore, we would conclude that any differences in *C. difficile* prevalence in the wastewater samples between the two populations would be attributable to swine exposure. This is the first study to assess the risk of occupational exposure to swine and *C. difficile* infection. Elsewhere, it has been shown that there is an increased risk of occupational exposure to *C. difficile* for health care workers in a clinical setting (4, 9, 44). There was also no difference in the prevalence of *C. difficile* in the human wastewater samples compared across unit types (i.e., farrow-to-finish units versus grower-to-finisher units). Units with a high prevalence in swine did not necessarily have

a high prevalence in humans. To illustrate, unit 3, which had the lowest prevalence among the farrow-to-finish units for swine, had the second highest prevalence for the human wastewater samples (Table 1). This provides further evidence that there is little or no occupational risk of *C. difficile* infection arising from direct exposure to swine.

A significantly ($P < 0.05$) increased prevalence of *C. difficile* in wastewater was identified during the spring; this included the months of March, April, and May. Seasonal trends in bacterial carriage are not unusual, and there have been conflicting results regarding seasonal trends of *C. difficile* in the hospital setting (29, 37, 46). Differences in seasonal trends noted among previous studies may be due to variations in the study populations or geography. Importantly, differences in seasonal trends between host species in this study may be due to exposure to environmental sources of *C. difficile* and differences in these environments among the host species as well.

The majority of both the human wastewater and swine samples were of toxinotype V (Table 2). The finding of toxinotype V as the dominant toxinotype in the swine isolates is consistent with other reports (14, 35). Toxinotype V is not a strain that has been recognized as a major cause of disease in hospitals (34); however, it has been isolated from humans, and some studies have suggested that the rate of toxinotype V isolation from humans is increasing (21). One of the reasons we may have identified a high percentage of toxinotype V isolates among the human samples is because we sampled fecal materials arising from asymptomatic individuals, rather than hospitalized patients. It has been suggested that certain strains of *C. difficile* may be responsible for community-acquired infection (17), and these may be the strains identified more commonly among the general public.

The two most common PFGE patterns identified among the swine and human isolates were NAP7 and a NAP7 variant. The results from the swine isolates are consistent with other studies that have observed that the majority of isolates from swine are PFGE type NAP7 (21). Studies in human health care facilities have identified PFGE type NAP1 (ribotype 027) to be the virulent strain responsible for most of the recent outbreaks in North America and Europe (1, 20, 27). While no human clinical studies have made explicit mention of NAP7, studies have identified ribotype 078, toxinotype V isolates among human cases, and this is the strain most commonly associated with PFGE type NAP7 (17). Thus, the lack of reporting of NAP7 may simply reflect differences in typing preference across global regions and public health laboratory jurisdictions.

Other studies have also identified a high degree of similarity between human and swine strains of *C. difficile* (14, 21). The biggest difference between our study and previous studies is that both of our swine and human populations were contained within the same closed system. Previously published studies have compared human and swine strains that arose from different study populations, and often at different times, and this makes it difficult to interpret any association between *C. difficile* infection in humans and various potential food sources. Similar strain carriage between host species in the same study population provides some evidence for possible transmission between species; however, an equally plausible explanation would be a common environmental source. *C. difficile* spores can survive in the environment for long periods of time under adverse conditions (22). *C. difficile* may be a ubiquitous environmental contaminant, and the more places we look for it, the more places we will find it. The finding of anaerobic, Gram-positive bacteria in the environment is not uncommon. A study conducted in South Wales, United Kingdom, isolated *C. difficile* from various environmental sources, including rivers, lakes, oceans, and soil (2), and *Clostridium tetani* spores are abundant in the soil and environment, especially in areas surrounding human or animal habitations (16).

This study provides evidence that occupational and food-borne exposures are less likely to be sources of community-acquired *C. difficile* infections than previously suggested (42). Similar strain carriage identified between the two host species suggests that a common environmental source may be an equally viable hypothesis. Further research is needed to investigate the possible sources of community-acquired *C. difficile* infections in humans and the component causes needed to propagate the strains associated with clinical disease.

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Proprietary or brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product, and/or exclusion of others that may be suitable.

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