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Clostridioides difficile on Ohio swine farms (2015): A comparison of swine and human environments and assessment of on-farm risk factors

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Abstract

Swine are known reservoirs for *Clostridioides difficile*, formerly known as *Clostridium difficile*, and transmission from swine to human farm workers is strongly suggested by previous studies. This cross-sectional study evaluated the potential role of farm environmental surfaces, including those in worker breakrooms and swine housing areas, in the possible transmission of *C. difficile* from swine to farm workers. Environmental surfaces and piglet faeces at 13 Ohio swine farms were sampled in 2015. Typical culturing techniques were performed to isolate *C. difficile* from samples, and amplification of toxin genes (*tcdA*, *tcdB* and *cdtB*) and PCR-ribotyping were used to genetically characterize recovered isolates. In addition, sequencing of toxin regulatory gene, *tcdC*, was done to identify the length of identified deletions in some isolates. A survey collected farm-level management risk factor information. *Clostridioides difficile* was recovered from all farms, with 42% (188/445) of samples testing positive for *C. difficile*. Samples collected from all on-farm locations recovered *C. difficile*, including farrowing rooms (60%, 107/178), breakrooms (50%, 69/138) and nursery rooms (9%, 12/129). Three ribotypes recovered from both swine and human environments (078, 412 and 005) have been previously implicated in human disease. Samples taken from farrowing rooms and breakrooms were found to have greater odds of *C. difficile* recovery than those taken from nursery rooms (OR = 40.5, OR = 35.6, $p < .001$ respectively). Farms that weaned ≥23,500 pigs per year had lower odds of *C. difficile* recovery as compared to farms that weaned fewer pigs (OR = 0.4, $p = .01$) and weekly or more frequent cleaning of breakroom counters was associated with higher odds of *C. difficile* recovery (OR = 11.7, $p < .001$). This study provides important insights into the presence and characterization of *C. difficile* found in human environments on swine farms and highlights how these areas may be involved in transmission of *C. difficile* to swine farm workers and throughout the facility.

KEYWORDS

biosecurity, breakroom, *Clostridioides difficile*, farm worker, swine, zoonotic transmission

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1 | INTRODUCTION

Clostridioides difficile is one of the most well-known human healthcare-associated infections (HAIs) in the United States, with an estimated 430,000 cases of *C. difficile* infection (CDI) in 2011 (Lessa et al., 2015). In recent decades, CDI cases without healthcare exposures, termed community-associated CDI (CA-CDI), have become increasingly common (McDonald et al., 2007). Due to this shift in the epidemiology of the disease, investigation into the sources of *C. difficile* in the community, as well as risk factors for CA-CDI, have become important areas of research. Animals are a possible source of *C. difficile* in the community, and swine are one of the species most frequently associated with carriage of the bacterium (Janezic et al., 2014). Swine, particularly neonatal piglets, can exhibit diarrheal signs or carry the bacteria subclinically. Prior studies estimate *C. difficile* carriage in healthy piglets less than 2 weeks old to range from 68% to 94%, and significantly decrease to 0%–8% in slaughter-age pigs (Arruda, 2014; Schneeberg et al., 2013).

The potential for zoonotic transmission of *C. difficile* from swine to humans, especially humans that have close contact with swine or their environment, has been documented by several studies (Keessen, Harmanus, Dohmen, Kuijper, & Lipman, 2013; Knetsch et al., 2014, 2017). For instance one study recovered *C. difficile* from 21% (18/70) of faecal samples from swine farm workers who had daily to weekly contact with swine, with the human and swine isolates determined to be genetically related, and identified the frequency of swine contact to have a positive association with *C. difficile* carriage (Keessen et al., 2013).

Despite the high frequency of *C. difficile* colonization in swine and potential for zoonotic transmission from swine, to-date there has been limited research on likely mechanisms for transmission from swine to swine farm workers, such as *C. difficile* environmental surface contamination on farms. One study documented the increase in *C. difficile* contamination of swine farrowing pens after the introduction of newborn piglets, highlighting the role of piglet faecal shedding in the contamination of the surrounding environment (Hopman et al., 2011).

Unlike other non-spore-forming pathogens found on farms, *C. difficile* spores are extremely resistant to the non-sporicidal disinfectants commonly used in swine farm settings, enabling spores to survive on hard surfaces for up to 5 months (Fekety et al., 1981). Many farms have within-facility worker breakrooms where high-risk hand-to-mouth activities occur (e.g., eating), personal protective equipment is often not worn and farm biosecurity protocols may be inadequate to limit the contamination of these dedicated human areas by environmental pathogens originating from other areas in the facility. Given the likely high environmental presence of *C. difficile* in swine-contact areas (i.e. housing, corridors) on farms, the ability of *C. difficile* to persist in the environment for extended periods, and the close proximity of worker breakrooms to swine-contact areas, the possible contamination of breakroom surfaces may introduce numerous opportunities for transmission of *C. difficile* to swine farm workers.

Impacts

- This study characterizes *C. difficile* on a sample of Ohio swine farms, comparing isolates recovered from young swine housing areas and on-farm worker breakrooms by PCR-ribotype and toxin profile (*tcdA*, *tcdB*, *cdtB*, *tcdC*)
- Highlights the widespread presence of *C. difficile* on common surfaces in swine farm breakrooms, an area on farms which may pose a threat to farm worker health and in which there has been limited prior research
- Identifies farm characteristics associated with the recovery of *C. difficile*, which may serve as avenues for future studies that aim to improve farm biosecurity and reduce on-farm transmission of pathogens

The objectives of this study were to compare the recovery of *C. difficile* and distribution of PCR-ribotypes in the swine and human environments from a sample of Ohio swine farms, and to identify factors associated with the recovery of *C. difficile* on farms, with the goals of characterizing the presence of *C. difficile* in an area on the swine farm that has not been highly studied but could present an unforeseen risk to farm worker health.

2 | MATERIALS AND METHODS

2.1 | Study design and farm recruitment

A convenience sample of swine farms located in Ohio that raised pigs from farrowing with an expected litter on their farm in the coming 5 months were approached and invited to participate. A cross-sectional study was conducted, and research personnel collected environmental samples from human and swine environments on farms and faecal samples from pigs housed in nursery and farrowing rooms on farms from May through August 2015.

2.2 | Sample collection

Between 10 and 15 samples were collected from each of three farm environments (i.e. farrowing, nursery, breakroom) on each farm. Samples were collected from surfaces in swine environments (i.e. farrowing, nursery) with direct swine contact, including pen gates (n = 5) and pen floors (n = 5), and fresh piglet faeces collected from pen floors (n = 5). Between 9 and 13 samples were collected from the human environment (i.e. breakroom) on each farm. Most farms had designated worker breakrooms, but for those that did not, the breakroom was identified as the place on-site where most employees most frequently ate lunch. Due to differences in breakrooms, objects/areas sampled in the human environments were not always the same between farms. However, common surfaces, like counters, refrigerators and doorknobs, were sampled in different breakrooms when possible.

All sampling materials were pre-packaged into sterile Whirl-Paks® (Nasco) using clean gloves in a laboratory biosafety cabinet prior to each farm visit and returned to the same packaging after collection. Two members from the research team conducted all sampling. Depending on the surface, either gauze cloths or electrostatic pads (Swiffer®; Proctor & Gamble Company) were used for sample collection, as in similar studies (Faires, Pearl, Berke, Reid-Smith, & Weese, 2013; Hopman et al., 2011). Due to difficulties with electrostatic pads falling apart when sampling rough surfaces, such as swine-area gates and pen floors, gauze was used to sample these areas, whereas electrostatic pads were used to sample smoother surfaces in worker breakrooms. One team member rubbed a gauze cloth, moistened with ~5 ml of sterile skim milk, over pre-determined areas in the swine environments. Piglet faecal samples were collected from the floors of pens using the same method. Electrostatic pads were rubbed over identified areas/objects in the human environment. One control sample was collected in the human environment on each farm by removing an electrostatic pad in the breakroom and immediately returning it to its Whirl-Pak®.

2.3 | Culturing techniques

All samples were immediately refrigerated upon arrival at The Ohio State University after each farm visit and processed within 24 hr of collection. The sample cloth/pads were enriched in *C. difficile* moxalactam norfloxacin (CDMN) selective enrichment broth with 0.1% sodium taurocholate, as previously described (Weese, Avery, Rousseau, & Reid-Smith, 2009). Gauze and electrostatic pad samples were fully saturated with 9 and 12 ml of CDMN broth, respectively, and incubated in their collection Whirl-Paks® at 37°C for 7 days. Following enrichment, 2 ml of the sample underwent an alcohol shock, was centrifuged for 10 min at 3,800 g, and the resulting pellet was transferred onto CDMN agar with 7% horse blood. These plates were incubated anaerobically at 37°C for 48–72 hr. A single colony with typical morphology was subcultured onto Columbia blood agar (CBA) with 5% horse blood and incubated anaerobically at 37°C for 48 hr. Resulting colonies were evaluated by gram stain, colony morphology, characteristic odour and L-proline aminopeptidase activity as previously described (Knight, Squire, & Riley, 2014; Weese et al., 2009); colonies that were suspected to be *C. difficile* underwent confirmatory testing.

2.4 | Molecular confirmation and characterization

DNA was extracted from fresh colonies grown on blood agar plates, using a Qiagen DNeasy Blood & Tissue Kit (Qiagen). Isolates were confirmed as *C. difficile* through PCR amplification of the triose phosphate isomerase (*tpi*) gene (Table 1), as previously described (Fry, Thakur, Abley, & Gebreyes, 2012; Lemee et al., 2004). Isolates confirmed as *C. difficile* were tested for the presence of *tcdA*, *tcdB*, *cdtB* and *tcdC* to characterize toxigenic potential. *TcdA*- and *tcdB*-specific primers (Table 1) were used in a multiplex PCR, as described previously (Lemee et al., 2004). *CdtB*-specific primers (Table 1) were used to identify the presence of the binary toxin gene according to a previously described protocol (Stubbs et al., 2000). Samples that were negative for the toxins or had a toxin profile inconsistent with the identified ribotype were rechecked using the multiplex method by Persson, Torpdahl, and Olsen (2008). All reactions and agarose gels were run alongside a reaction with DNA from strain ATCC 9689 as a positive control, and a reaction without DNA as a negative control. All isolates underwent capillary electrophoresis PCR-ribotyping as previously described (Fawley et al., 2015). International designations (i.e. ribotype 078) were used for strains where reference strains were available; otherwise new ribotype designation numbers were assigned.

Isolates were screened for the presence of the *tcdC* toxin negative regulator gene and a previously identified 39-bp deletion in the gene (Fry et al., 2012). PCR was performed using *tcdC*-specific primers (Table 1) that produced an amplicon < 364-bp if the regulator sequence contained a deletion, following a previously described protocol, with a modified annealing temperature of 53°C (Fry et al., 2012). The PCR product was run on a 1.5% agarose gel for 2 hr to differentiate products with a deletion from the fully intact 364-bp product of the control. A subset of the isolates identified as containing a *tcdC* deletion (n = 33) were Sanger sequenced (GENEWIZ LLC) to determine the size of the deletion.

A 5-min self-administered survey for farm managers was developed by the research team to obtain information about individual

TABLE 1 List of PCR primers used in this study

Gene	Primer		Amplicon (bp)	References
	Name	Sequence (5′–3′)		
tpi	tpi-F	AAAGAAGCTACTAAGGGTACAAA	230	Lemee et al. (2004)
	tpi-R	CATAATATTGGGTCTATTCCTAC		
tcdA	tcdA-F	AGATTCCTATATTTACATGACAATAT	369	Lemee et al. (2004)
	tcdA-R	GTATCAGGCATAAAGTAATATACTTT		
tcdB	tcdB-F	GGAAAAGAGAATGGTTTTATTAA	160	Lemee et al. (2004)
	tcdB-R	ATCTTTAGTTATAACTTTGACATCTTT		
cdtB	cdtBpos	CTTAATGCAAGTAAATACTGAG	510	Stubbs et al. (2000)
	cdtBrev	AACGGATCTCTTGCTTCAGTC		
tcdC	tcdC-F	TGAGGAGGTCATTTCTAACCA	≤364	O'Shaughnessy
	tcdC-R	TCCAGACACAGCTAATCTTATTTGC		

farm characteristics and biosecurity practices, focusing on potential mechanisms and risk factors for *C. difficile* transmission (survey available from the corresponding author). Surveys were provided to farm managers, one per farm, in both English and Spanish, along with a letter outlining the objectives of the study, and returned to the research team by mail. All materials and protocols were approved under the University Institutional Review Board (#2015H0027).

2.5 | Statistical analysis

Survey responses were uploaded into Stata 14.2 (StataCorp) and coded in a binary fashion. For open-ended questions, the responses were converted into binary variables. Questions regarding frequency of cleaning practices were dichotomized ("weekly or more frequent" or "less than weekly"), based on distribution of the data. A binary variable for herd size was created based on the median reported herd size of enrolled farms, referred to hereafter as small-herd (<23,500 pigs weaned per year) and large-herd farms (≥23,500 pigs weaned per year). Farm-level survey variables were merged with *C. difficile* isolation data.

Descriptive statistics were calculated for each variable. Mixed effects logistic regression modelling with random intercept was performed in Stata (*xtmelogit*) to determine associations between reported farm characteristics and biosecurity protocols and the isolation of *C. difficile* from environmental and faecal samples, while accounting for clustering of samples at the farm level. To ensure adequate variability in responses, variables were ineligible for analysis if the discordance in responses was < 15%, meaning that the binary response frequency for a variable had to be at least 1:5. A total of 21 variables were evaluated, including on-farm sample location (i.e. farrowing, nursery or breakroom).

Univariable analyses with *C. difficile* recovery as the dependent variable and variables from the survey as independent predictors, were completed. Predictors with $p < .20$ in the univariable models were eligible for inclusion in a multivariable model. Using a backwards selection process, all predictors that met the significance criterion were evaluated, and variables were removed until all remaining predictors were significant ($p < .05$). After significant main effects were determined, biologically plausible confounders were re-introduced, and retained in the final model if there was a change >20% of any model coefficient.

3 | RESULTS

From 13 Ohio swine farms, 445 total samples were collected from farrowing rooms (n = 178), nursery rooms (n = 129) and worker breakrooms (n = 138). Eight farms had both a nursery and farrowing room, one farm only a nursery room and four farms only farrowing rooms. All farms had a location that served as a worker breakroom. Twelve of the 13 farms (92%) submitted a completed survey. *Clostridioides*

TABLE 2 Distribution of *C. difficile* recovery across on-farm locations and sample sources on Ohio swine farms (N = 13)

On-farm location	Sample source	No. samples collected	No. *C. difficile* isolates recovered (%)
Overall[a]		445	188 (42)
Farrowing	All[b]	178	107 (60)
	Floor	62	36 (58)
	Gate	61	34 (56)
	Piglet Faeces	55	37 (67)
Nursery	All[c]	129	12 (9)
	Floor	43	2 (5)
	Gate	46	3 (7)
	Piglet Faeces	40	7 (18)
Breakroom	All[d]	138	69 (50)
	Floor	13	9 (69)
	Counter/Table	18	8 (44)
	Refrigerator	29	18 (62)
	Door/Knob	22	10 (46)
	Sink	22	12 (55)
	Cabinet	13	8 (62)
	Microwave	13	4 (31)
	Other[e]	8	0 (0)

[a]Overall recovery ranged from 4%–68% (median: 48%) among farms.
[b]Recovery in farrowing room ranged from 23% to 100% (median: 53%) among farms.
[c]Recovery in nursery rooms ranged from 0%–27% (median: 0%) among farms.
[d]Recovery in breakrooms ranged from 10%–90% (median: 59%) among farms.
[e]Other surfaces included toilets (n = 5), a phone, a toaster and a water cooler.

difficile was recovered from all farms (*n* = 13), with an overall recovery of 42% (188/445) from all samples (Table 2). At the farm-level, recovery ranged from 4%–68% (median 48%). *Clostridioides difficile* was isolated from samples collected from all on-farm locations, including farrowing rooms (60%, 107/178), breakrooms (50%, 69/138) and nursery rooms (9%, 12/129) (Table 2). Within swine housing areas, *C. difficile* was frequently recovered from piglet faecal samples and environmental surfaces [farrowing: faecal (37/55; 67%), environmental (70/123; 57%); nursery: faecal (7/40; 18%), environmental (5/89; 7%); Table 2]. Across all worker breakrooms, *C. difficile* was cultured in the greatest proportion from breakroom floors (9/13, 69%), refrigerators (18/29, 62%), cabinets (8/13, 61%) and sinks (12/22, 54%) (Table 2).

Four of 20 survey variables were eligible for the multivariable model ("farm type", "unusual pig diarrhoea observed in the past two weeks", "weekly or more frequent cleaning of breakroom counters" and "weekly or more frequent cleaning of human bathrooms"); Table 3. Notably, "sample source" (i.e. environment, piglet faeces) was not found to be a significant predictor of *C. difficile* recovery. In the multivariable model, "farm type" was not significant. From the final multivariable model, the odds of *C. difficile* recovery were significantly greater for samples collected from farrowing rooms and breakrooms than those collected from nursery rooms (OR = 40.5, OR = 35.6 respectively; $p < .001$) (Table 4). Samples from farms that reported weekly or more frequent cleaning of breakroom counters and recent unusual pig diarrhoea had greater odds of recovering *C. difficile* than those that did not report these practices and findings (OR = 11.7, $p < .001$; OR = 3.0, $p = .003$ respectively; Table 4). In contrast, large-herd farms had a significantly lower odds of *C. difficile* recovery than small-herd farms (OR = 0.4, $p = .01$; Table 4).

Ninety percent (169/188) of all isolates were classified as toxigenic, and many isolates (142/188, 76%) contained all three toxin genes (Table 5). A total of 181 *C. difficile* isolates were ribotyped and six different ribotypes identified (078, 596, 412, 005, PR22379 and PR22380). Seven isolates were unable to be ribotyped because they were unable to be cultured from preserved specimens. Ribotype 078 was recovered from both the human and a swine environment on >75% of farms (Table 5). This ribotype was recovered from all *C. difficile*-positive surface types sampled in breakrooms. Three other ribotypes identified (412, 596 and 005) were recovered from a swine area and the associated breakroom on at least one farm, each (Table 5). Refrigerators, cabinets and sinks carried the greatest number of ribotypes among all surface types sampled in breakrooms (Table 5). A truncation in *tcdC* was detected in 74% (139/188) of all isolates recovered, and all isolates in the subset sequenced (*n* = 33) were confirmed to have a 39-bp deletion and identified as ribotype 078.

4 | DISCUSSION

We noted that breakrooms, where workers likely have high-risk oral transmission behaviours (e.g., food and drink consumption), were highly contaminated with toxigenic *C. difficile*, with recovered isolates similar to those recovered from areas that house pre-weaned swine. Studies have suggested swine workers to be at increased risk for *C. difficile* colonization, but limited studies to-date have explored the possible sources of *C. difficile* acquisition within the farm environment (Keessen et al., 2013). This study provides an evaluation of the widespread presence of *C. difficile* in the human environment on swine farms, suggesting potential transmission from adjacent, and highly contaminated, swine-populated rooms. Research on the contamination of worker breakrooms with *C. difficile* on farms is an area that may have important findings for public health and swine and worker safety, and in which there is very limited prior research.

In this study, *C. difficile* was recovered from all farms (*n* = 13), with an overall high recovery (42.3%). Although *C. difficile* in the breakroom may originate from sources other than swine on the farm, including food items, human faeces, water and soil, the proportion of *C. difficile*-positive samples was higher than expected, and ribotypes different than what would be expected if these non-swine sources were solely responsible for the observed contamination. Previous studies that also sampled with electrostatic pads recovered *C. difficile* from a drastically lower proportion of household (5.3%) and hospital (6.4%) surfaces (Faires et al., 2013; Weese, Finley, Reid-Smith, Janecko, & Rousseau, 2010). The previous study that used identical methods to assess *C. difficile* recovery from surfaces in 84 households with pets (i.e. dogs, cats), recovered *C. difficile* from a very low number of household surfaces (5%, 44/836), including floors, kitchen sinks, kitchen countertops, refrigerators and toilets (Weese et al.,2010). The greater proportion of *C. difficile* isolation in this study suggests that the presence of swine, and neonates especially, in the farm setting is likely the cause of the increased contamination of surfaces with *C. difficile* in farm breakrooms, although cleaning practices and other differences cannot be discounted. The contamination of the on-farm human environment reported in this study highlights an area of the farm where workers are exposed to *C. difficile*, but where they may not practice the same biosecurity measures as when they are working in swine areas where *C. difficile* contamination is expected.

As reported in other studies, we noted a large difference between the recovery of *C. difficile* in farrowing (60%) and nursey rooms (9%) (Fry et al., 2012; Keessen, Donswijk, et al., 2011; Keessen, Gaastra, & Lipman, 2011). These data strongly support swine as the source of *C. difficile* contamination in the swine areas, as has been suggested by previous literature (Hopman et al., 2011). In both swine rooms, *C. difficile* was recovered most often from piglet faeces, with gates and floors of pens exhibiting a notably lower presence than faecal samples. Workers and surfaces or objects (e.g. worker boots) that have direct or indirect contact with swine faeces may easily become mechanical vehicles for transmission of *C. difficile* between locations on the swine farm, including between swine housing areas and the breakroom.

In a similar proportion to farrowing rooms, *C. difficile* was frequently isolated from breakroom surfaces (50%), and both farrowing rooms and breakrooms had significantly greater odds of *C. difficile*

TABLE 3 Univariable analysis of predictors of *C. difficile* recovery on 12 Ohio swine farms using a mixed effects logistic regression model

Predictors	Response	No. farms	No. samples *C. difficile*+ (%)	No. samples *C. difficile*− (%)	Odds ratio (95% confidence interval)	*p*-Value
Operation style	Single site	3	161 (61)	70 (59)	Referent	.87
	Multiple sites	9	101 (39)	48 (41)	0.9 (0.3, 2.6)	
Farm type[a]	Farrow to wean	5	121 (65)	86 (51)	Referent	.12
	Farrow to finish	6	65 (35)	83 (49)	0.6 (0.3, 1.1)	
AIAO production method used in farrowing room[b]	No	2	181 (59)	26 (52)	Referent	.58
	Yes	9	124 (41)	24 (48)	0.8 (0.3, 2.0)	
Recent unusual pig diarrhoea observed (within past 2 weeks)[a]	No	9	57 (48)	174 (67)	Referent	.09
	Yes	3	62 (52)	87 (33)	2.2 (0.9, 5.4)	
Animal on farm previously diagnosed with *C. difficile* by veterinarian[b]	No	9	38 (48)	193 (64)	Referent	.2
	Yes	2	41 (52)	108 (36)	2.1 (0.7, 6.5)	
Shower before entering/exiting barn (SISO)[b]	No	4	155 (66)	58 (55)	Referent	.24
	Yes	7	80 (34)	48 (45)	0.6 (0.2, 1.4)	
Virkon used to clean farrowing[b]	No	6	96 (57)	111 (59)	Referent	.87
	Yes	5	72 (43)	76 (41)	1.1 (0.5, 2.2)	
Bleach used to clean farrowing[b]	No	7	68 (52)	139 (62)	Referent	.31
	Yes	4	62 (48)	86 (38)	1.4 (0.7, 2.9)	
Tek-Trol® used to clean farrowing[b]	No	6	70 (59)	137 (58)	Referent	.91
	Yes	5	48 (41)	100 (42)	1.0 (0.5, 2.1)	
Synergize® used to clean farrowing[b]	No	6	88 (60)	119 (57)	Referent	.87
	Yes	5	59 (40)	89 (43)	0.9 (0.5, 1.9)	
Virkon® used to clean nursery[b]	No	7	51 (65)	135 (64)	Referent	.87
	Yes	2	27 (35)	77 (36)	0.9 (0.2, 3.6)	
Bleach used to clean nursery[b]	No	7	47 (72)	139 (62)	Referent	.21
	Yes	2	18 (28)	86 (38)	0.4 (0.1, 1.7)	
Tektrol used to clean nursery[b]	No	6	44 (65)	142 (64)	Referent	.58
	Yes	3	24 (35)	80 (36)	1.4 (0.4, 4.8)	
Synergize used to clean nursery[b]	No	4	112 (65)	74 (63)	Referent	.46
	Yes	5	60 (35)	44 (37)	0.7 (0.2, 2.0)	
Swine area counters cleaned weekly or more frequently[b]	No	4	78 (60)	72 (68)	Referent	.66
	Yes	4	52 (40)	34 (32)	1.2 (0.5, 3.0)	
Swine corridors cleaned weekly or more frequently	No	8	72 (56)	159 (63)	Referent	.47
	Yes	4	56 (44)	93 (37)	1.4 (0.6, 3.6)	
Breakroom counters cleaned weekly or more frequently[a]	No	3	168 (58)	45 (85)	Referent	.17
	Yes	8	120 (42)	8 (15)	2.1 (0.7, 6.2)	
Bathroom cleaned weekly or more frequently[a]	No	4	91 (54)	88 (66)	Referent	.12
	Yes	5	77 (46)	45 (34)	2.1 (0.8, 5.1)	
Sample source	Environment	–	144 (77)	206 (80)	Referent	.41
	Faeces	–	44 (23)	51 (20)	1.2 (0.8, 2.0)	
Sampled from farrowing room[a]	No	–	71 (40)	186 (70)	Referent	<.001
	Yes	–	107 (60)	81 (30)	3.5 (2.3, 5.3)	
Sampled from breakroom[a]	No	–	69 (50)	188 (61)	Referent	.01
	Yes	–	69 (50)	119 (39)	1.8 (1.1, 2.7)	
Herd size[b]	<23,500 pigs weaned per year	5	141 (60)	90 (62)	Referent	.87
	≥23,500 pigs weaned per year	5	94 (40)	55 (38)	1.1 (0.4, 1.9)	

[a] $p < .20$, tested in the multivariable model.
[b] One or more farm responses missing.

TABLE 4 Multivariable analysis of predictors of *C. difficile* recovery on 12 Ohio swine farms using a mixed effects logistic regression model (n = 380 samples)

Predictor	Response	Adjusted odds ratio (95% confidence interval)[a]	*p*-Value[a]
Sample location	Nursery	Referent	
	Breakroom	35.6 (11.2, 113.1)	<.001
	Farrowing	40.5 (13.1, 125.5)	<.001
Breakroom counters cleaned weekly or more frequently	No	Referent	<.001
	Yes	11.7 (3.4, 39.6)	
Recent unusual pig diarrhoea observed (within past 2 weeks)	No	Referent	.003
	Yes	3.0 (1.4, 6.3)	
Herd size	<23,500 pigs weaned per year	Referent	.01
	≥23,500 pigs weaned per year	0.4 (0.2, 0.8)	

[a]Controlling for "bathroom cleaned weekly or more frequently".

TABLE 5 Source and molecular characterization of *C. difficile* isolates (n = 188) recovered from the farm environmental and piglet faecal samples on 13 Ohio swine farms

Ribotype	No.	Farm ID	On-farm location	Sample source	*tcdA*	*tcdB*	*cdtB*	*tcdC*
078	141	1,3,5,6,7,8,9,11,12,13	Farrowing	Piglet faeces, floor, gate	Pos.	Pos.	Pos.	Intact (3) Truncation (138)
		5,10,12,13	Nursery	Piglet faeces, floor, gate				
		1,2,3,4,5,6,7,8,9,11,12,13	Breakroom	Floor, counter/table, refrigerator, door/knobs, sink, cabinet, microwave				
412	21	4	Farrowing	Piglet faeces, floor, gate	Pos.	Pos.	Neg.	Intact
		4,5	Breakroom	Counter/table, refrigerator, door/knobs, cabinet, sink, microwave				
596	11	2,5	Farrowing	Piglet faeces, floor, gate	Neg.	Neg.	Neg	Neg.
		2	Breakroom	Floor, refrigerator				
005	6	10	Farrowing	Piglet faeces, floor	Pos.	Pos.	Neg.	Intact (6)
		13	Nursery	Piglet faeces				
		10	Breakroom	Cabinet				
PR22379	1	8	Breakroom	Sink	Pos.	Pos.	Pos.	Truncation
PR22380	1	12	Farrowing	Gate	Neg.	Neg.	Neg.	Neg.
NRT[a]	7	5	Nursery	Piglet faeces, gate	Neg.	Neg.	Neg.	Neg.
		3,5	Breakroom	Door/knobs, cabinet				

[a]Not PCR-ribotyped; unable to be cultured from preserved specimens.

recovery as compared to nursery rooms. Breakroom cleaning practices may have contributed to the high *C. difficile* contamination of the breakrooms if sporicidal disinfectants were used improperly or not at all. Unexpectedly, the self-reported more frequent cleaning of breakroom counters was significantly associated with a higher odds of overall *C. difficile* recovery than less frequent cleaning. This association may have been driven by farms with higher *C. difficile* recovery cleaning their breakrooms more often in response to prior

or current issues with infectious diseases (including *C. difficile*) or disinfecting breakroom surfaces using products or methods ineffective in the killing or removal of spores.

A comparison of the ribotypes recovered in this study further suggest the important role swine housing environments may play in the *C. difficile* contamination of adjacent worker breakrooms. The same *C. difficile* ribotypes recovered from most farm breakrooms were also recovered from at least one swine environment on those same farms. This finding was perhaps unsurprising, as there was minimal physical separation between these areas (e.g., breakrooms were often only separated from the swine environment by a door or less), heavily relying on procedural separation and related practices (e.g., use of personal protective equipment, hand hygiene) to reduce the movement of pathogens from one area to the other.

Three ribotypes identified in the swine and breakroom environments, 078, 005 and 412, have been previously associated with human CDI (Cheng et al., 2016; Fawley et al., 2016). In this study, ribotype 078 accounted for the majority of isolates recovered. Ribotype 078 is highly associated with CA-CDI human infections in the United States, Taiwan, the Netherlands and Canada (CDC, 2013; Goorhuis et al., 2008; Hung et al., 2016; Mulvey et al., 2010; Rupnik, 2010; Solomon et al., 2013). Furthermore, specifically *C. difficile* ribotype 078 with a 39-bp deletion in *tcdC*, cultured from swine faeces in previous literature as well as in this study, has also been previously implicated in human CDI (Fry et al., 2012; Hung et al., 2016). A 39-bp deletion in *tcdC* has been used as a "surrogate marker" to identify a hypervirulent strain of *C. difficile* ribotype 027 (i.e. BI/NAP1/027), which is likely caused by a single-nucleotide mutation at position 117 in the gene, resulting in a premature stop codon and a truncated gene product (Curry et al., 2007; Sloan, Duresko, Gustafson, & Rosenblatt, 2008). It is possible that this deletion may mark a similar potential for hypervirulence among isolates of ribotype 078, although the relevance of the deletion in this ribotype is still being explored (Persson et al., 2008). Ribotype 005 has also been implicated in human infections, identified through national surveillance systems in many countries, including Germany, the United Kingdom and Australia, and ribotype 412 has been cultured from patients with CDI in Italy (Cheng et al., 2016; Fawley et al., 2016; Reil et al., 2011; Sisto et al., 2014). As such, identifying ribotypes 078, 005 and 412 in breakrooms in this study is a potential public health concern.

Surprisingly, large-herd farms had significantly lower odds of *C. difficile* recovery as compared to farms with fewer swine. This association is contradictory to associations commonly found between farm herd-size and the prevalence of other highly studied enteric pathogens (e.g., *Salmonella*, *Campylobacter*, *Escherichia coli* O157:H7) (Adesiyun et al., 2014; Guerin et al., 2007; Worley et al., 2017). It may be that the swine farms in this study with larger herds had more stringent hygiene and biosecurity protocols in place or greater worker adherence to such protocols than those with smaller herds. In this study, antibiotic use on farms, biosecurity practices, including shower-in-shower-out (SISO) protocols and all-in-all-out (AIAO) production methods in both farrowing and nursery rooms, were not found to be significantly associated with the recovery of *C. difficile* due to the small number of enrolled farms and subsequent low variability in these self-reported factors between farms. Although the relationships between specific personal worker hygiene practices and *C. difficile* recovery were not addressed by this research, the transmission of pathogens from the pre-weaned swine to the human environment may be disrupted through more consistent use of PPE and handwashing by workers.

A limitation of this study is the utilization of a convenience sample of swine farms, thus the results of this study may not be generalizable to farms outside the study group. Additionally, selection bias may have influenced our results if farms that were less-impacted by *C. difficile* were more willing to participate in this study. The recovery of *C. difficile* in swine areas may be underestimated, as electrostatic pads are the preferred and likely more sensitive method for sampling surfaces for pathogens as compared to moistened gauze cloths (Faires et al., 2012; Ruple-Czerniak, Bolte, Burgess, & Morley, 2014). Despite this limitation, the pattern and frequency with which *C. difficile* was found in swine areas, sampled with moistened gauze, was comparable to the findings of previous studies (e.g. proportion of *C. difficile* recovered from faeces of swine of similar ages; Arruda, 2014; Schneeberg et al., 2013). Although, the modelling of *C. difficile* recovery was performed at the sample level, allowing for a large sample size (n = 445), the hierarchical nature of the data with such a low number of enrolled farms and low variability in farm-level variables likely limited the study power. Furthermore, although the survey response was high (92.3%, 12/13), high non-response to individual questions lead to the omission of variables when modelling predictors of *C. difficile* recovery.

Despite these limitations, this study serves as an important step to guide future investigation into swine and farm worker risk for *C. difficile* colonization or CDI, as it identified likely important factors associated with *C. difficile* presence on farms that may serve as reasonable targets for future interventions. This study demonstrated *C. difficile* to be highly prevalent in swine farm breakrooms and identified *C. difficile* ribotypes present in both swine and the human environment on farms that are implicated in human CDI. Finally, this study provides a basis on which to further clarify the potential (and associated routes) for zoonotic transmission of *C. difficile* on swine farms.

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CONFLICTS OF INTEREST

No conflicts of interest exist.

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