Prevalence and characterization of *Clostridium difficile* from slaughter-age pigs

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Introduction

- *Clostridium difficile* is a cause of enteric disease in young piglets and can also be found in healthy pigs of varying ages.
- The finding of indistinguishable strains in food animals, food and people has raised concerns that potential that *C. difficile* might be a foodborne pathogen.
- Recent studies have indicated that the prevalence of *C. difficile* colonization can decrease dramatically as pigs age. Therefore, studies of pigs at the age of slaughter are required as part investigations into food contamination and foodborne disease risk, as well as for investigation of the epidemiology of C. dif*ficile* on pig farms.

Objectives

- To determine the prevalence of *C. difficile* shedding in slaughter-age pigs on farms across Canada, and to characterize recovered isolates.

Methods

- 45 swine farms from the 5 major swine producing provinces (Alberta (AB), Saskatchewan (SK), Manitoba (MB), Ontario (ON), Québec (QC)) were enrolled in conjunction with the Canadian Program for Integrated Antimicrobial Resistance Surveillance (CIPARS).
- Freshly passed fecal samples were collected from pens containing grower-finisher pigs close to the time of slaughter. A single sample was collected per pen. 10 10 samples were collected from most farm, representing 10 non-commingled pigs, however on some farms, 10 separate samples could not be collected.
- One gram of feces was inoculated into 9 ml *C. difficile* moxalactam-norfloxacin (CDMN) broth with 0.1% taurocholate. After 7d of incubation at 37^{0}C, an aliquot was alcohol shocked and inoculated onto CDMN agar.
- Isolates were characterized by ribotyping and PCR detection of *tcdA, tcdB and cdtB.* Ribotyping was performed. If the ribotype pattern was a recognized international ribotype, as determined thorough comparison with a reference strain, it was given the appropriate numerical designation (i.e. 078) Otherwise, an internal nomenclature was used. A representative of each ribotype was further characterized by toxinotyping, pulsed field gel electrophoresis (PFGE) and *tcdC* sequence analysis.

Results

- *C. difficile* was isolated from 30/435 (6.9%) pigs from 15/45 (33%) farms.
- On positive farms, *C. difficile* was isolated from 1-5 samples (median 1). That represented 10-100% of sampled pigs on individual farms.
- *C. difficile w*as recovered from more than 1 pig on 7 farms. On 3 of these farms, all isolates were the same ribotype (078). On the other farms, ribotype 078 plus 1-2 other ribotypes were identified.
- *C. difficile* infection was not diagnosed in any diseased pigs on any farm during the study period.
- There was a significant different in farm-level prevalence between provinces (*P*=0.002)(Table 1).
- Ribotype 078/NAP7/Toxinotype V predominated (Table 2). Two other toxinotype V ribotypes were also identified.
- One toxinotype V ribotype (S7) was indistinguishable on PFGE from NAP7 (Figure 1). The other toxinotype V strain had a PFGE pattern that suggested it was related to NAP7.
- Ribotype 078 possessed a 39bp deletion and upstream C184T nonsense truncating mutation, as did the other 2 toxinotype V strains. OVCAA possessed an 18 bp deletion but no mutations. The other toxigenic strains (MOH-S, S8) had an unaltered *tcdC.*
- Risk factors were not investigated because of the low prevalence.

Ribotype	n	TT	Toxin genes	Farms	Provinces
078	20	V	*tcdA, tcdB, cdtB*	12	AB, MB, ON, SK
S6	3	V	*tcdA, tcdB, cdtB*	2	SK
MOH-S	2	0	*tcdA, tcdB*	1	AB
S7	1	V	*tcdA, tcdB, cdtB*	1	SK
S8	1	NT	*tcdA, tcdB*	1	ON
OVCAA	1	0	*tcdA, tcdB*	1	AB
OVCJ	2	NT	None	2	MB

Table 2: Molecular characterization of *C. difficile* recovered from pigs. NT: not tested.

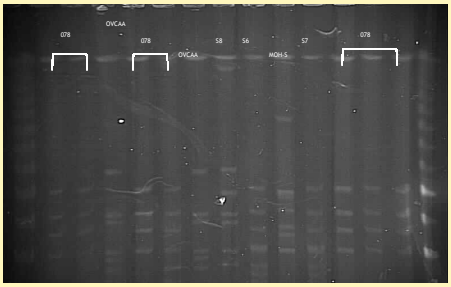

Figure 1: PFGE of selected C. *difficile* isolates. Ribotyping results are indicated at the top of the columns.

Discussion

- The relatively low prevalence of *C. difficile* is consistent with an age-related effect on the prevalence of colonization. A recent longitudinal study in Canada identified a cumulative prevalence of 96% in young piglets, with point prevalence declining from 74% on day 2 of life to 3.7% on day 62 (Weese et al, Anaerobe 2010). It appears that older pigs maintain a low prevalence.
- Studies investigating the epidemiology of *C. difficile* in pigs must consider the apparent effect of age. Comparison of studies involving different age groups should be avoided. Studies investigating food contamination must involve pigs close to the age of slaughter
- The predominance of ribotype 078 is consistent with other studies of food animals, and provides more support to the notion that this is a food-animal-associated strain.
- Ribotype 078 was not the only toxinotype V strain. Two other toxinotype V strains shared other characteristics of ribotype 078 and are presumably closely related. The public health relevance of non-078, toxinotype V strains is unclear but is likely similar to that of 078. The presence of these strains may indicate ongoing evolution of toxinotype V *C. difficile in* pigs.
- The regional variation was unexpected. It is unclear if that is a sampling artifact or evidence of true regional differences. Differences in management practices were not readily apparent, although specific investigation of farm level risk factors was not undertaken.
- The low prevalence in pigs of this age may have implications for assessment of foodborne contamination. The source of food contamination, along with the clinical relevance, requires further study.

Province	Pig prevalence	Farm prevalence
A	4/100 (4%)	3/11 (27%)
B	13/50 (26%)	5/5 (100%)
C	4/100 (4%)	4/10 (40%)
D	0/106 (0%)	0/11 (0%)
E	9/80 (11%)	3/8 (38%)
Total	30/436 (6.9%)	15/45 (33%)

Table 1: *C. difficile* prevalence by province.

Acknowledgements

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