

Evaluation of *Clostridium difficile* in dogs and the household environment

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SUMMARY

Clostridium difficile may be an emerging community-associated pathogen but little is known about its sources of exposure. This study evaluated *C. difficile* contamination in households and colonization of pets. *C. difficile* was isolated from 44/836 (5·3 %) sites in 26/84 (31 %) households. Ribotype 027 was the most common (25 %) environmental strain. *C. difficile* was isolated from 14/139 (10 %) dogs. Living with an immunocompromised individual was associated with *C. difficile* colonization in dogs. All toxigenic strains identified in pets have been isolated from humans in Ontario. *C. difficile* was isolated concurrently from dogs and the environment in four households, but in all cases canine and environmental ribotypes were different. *C. difficile* was relatively common in households, suggesting that exposure to this pathogen may be a regular event. There was no evidence that dogs are a significant source of household *C. difficile* contamination.

Key words: Anaerobic bacteria, *Clostridium*, domestic pets, zoonoses.

INTRODUCTION

Clostridium difficile is the most commonly diagnosed cause of antimicrobial- and hospital-associated diarrhoea in humans and there is evidence that *C. difficile* infection (CDI) may be an important emerging cause of community-associated (CA) disease, including serious disease in people traditionally considered to be at low risk. While *C. difficile* has been implicated as a cause of diarrhoea in dogs and cats [1–3], it can also be found in clinically normal animals. Colonization rates of up to 40 % have been found in populations

such as dogs and cats in veterinary clinics or shelters, with lower rates (0–10 %) in households [2, 4–6]. Further, *C. difficile* strains from dogs and cats are often indistinguishable from those found in humans [7, 8], which raises concern about the potential for zoonotic transmission. Canine and feline prevalence studies have often been limited in number and scope, and have typically relied on testing of single faecal samples. The potential role of the household environment as a source of *C. difficile* exposure has also not been investigated, along with the potential role of pets as source of contamination.

The objectives of this study were to evaluate *C. difficile* shedding by healthy dogs and cats in households using serial sampling, to identify factors associated with *C. difficile* shedding by dogs, to determine

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the prevalence of *C. difficile* in the household environment, to evaluate factors associated with environmental *C. difficile* contamination and to compare isolates from pets and the environment.

MATERIALS AND METHODS

A convenience sample of dog-owning households in southern Ontario, Canada was enrolled between October 2005 and May 2006 through advertising that targeted the University of Guelph, local veterinary conferences and meetings, and local veterinary clinics. Study personnel visited households and administered a questionnaire that evaluated the presence of other pets and the number of dogs in the household, the dog's diet, the presence of gastrointestinal illness in the dog in the previous 30 days, frequency of feeding and cleaning of food bowls, dog's activities and exposure to farm animals, and household information such as the presence of infants and immunocompromised (self-identified) individuals. Environmental sampling was performed at nine locations in each household: toilet, dog food bowl, refrigerator shelves, kitchen sink, kitchen counter, kitchen sink taps, dog eating area, main entry and general floor. Using a gloved hand, study personnel wiped a clean electrostatic cloth (Swiffer[®], Proctor and Gamble, USA) over the area to be sampled and vacuum bag contents were collected. Participants submitted freshly passed faecal samples from their pet(s) daily for 5 days.

Cloths were immersed in 50 ml *C. difficile* moxalactam norfloxacin (CDMN) broth with 0.1% sodium taurocholate and incubated aerobically at 37 °C for 7 days. Alcohol shock was then performed, followed by inoculation onto Columbia blood agar and incubation for 48–120 h at 37 °C in an anaerobic chamber. Colonies with the characteristic morphology, odour and Gram stain appearance were subcultured and confirmed as *C. difficile* by morphology and production of L-prolineaminopeptidase (Pro Disc, USA).

Stool samples were cultured using a similar enrichment protocol, using 200–300 mg faecal sample and 9 ml CDMN broth with 0.1% sodium taurocholate.

Isolates were characterized by ribotyping, toxin gene PCR and toxinotyping [9]. For ribotyping, international designations (i.e. ribotype 027) were used for strains where reference strains were available. Otherwise, internal alphabetic designations were used.

Categorical comparisons were performed using Fisher's exact test. Generalized estimating equations

were used to evaluate clustering of *C. difficile* at the household level. Due to the absence of clustering, exact logistic regression was used for the evaluation of continuous data. Multivariable analysis was not performed because of the sample size and prevalence. However, models with a potential confounder and a significant explanatory variable were used to test the impact of these confounders. Potential confounders included breed size (small, medium, large, mixed breeds), age (<1 year, 1–7 years, >7 years), gender (male vs. female) and neuter status. Confounders were identified when the odds ratio of the independent variable changed by more than 20% when the potentially confounding variable was added to the model. A *P* value of ≤0.05 was considered significant for all comparisons. The University of Guelph Research Ethics Board approved this study.

RESULTS

Eighty-four households containing one or more dogs were enrolled (mean 2.13, median 2.00, range 1–7). Dogs ranged in age from 6 months to 16 years (5.3 ± 3.5 years, mean \pm s.d.) and 56% were females. Cats were present in 26 (31%) of these households, but data were only included for households with a single cat (12 households) because in 14 other households multiple cats were present with a shared litter-box making it impossible to determine from which cat a fecal sample came.

C. difficile was isolated from 14/139 (10%, 95% CI 6.1–16.2) of dogs and 3/14 (21%, 95% CI 5.7–51.2) cats (*P*=0.19). Thirteen (93%) of the dogs and all of the cats that tested positive were only positive on one of the five samples. The only dog that had more than one positive sample shed *C. difficile* on days 1, 2, 3 and 5. Overall, *C. difficile* was isolated from 20/695 (2.9%) canine and 3/70 (4.3%) feline samples (*P*=0.21). Multiple colonized animals were identified in three households, two with *C. difficile* from a dog and a cat and one with *C. difficile* from two dogs. Because of the small number of cats from which *C. difficile* was isolated, subsequent data analysis only involved dogs.

Dogs living with a person self-identified as immunocompromised were 7.9 times (95% CI 1.0–53.55, *P*=0.02) as likely to shed *C. difficile* than other dogs while dogs allowed to run freely in a park were 3.3 times (95% CI 0.9–13.4, *P*=0.04) less likely to shed *C. difficile* as determined by univariate exact logistic regression. Breed size, age, sex and gender status were included individually as potential confounders. Only

Table 1. Source and molecular characteristics of toxigenic *C. difficile* isolated from dogs, cats and the household environment

Ribotype	<i>n</i>	Environmental site	Animals (<i>n</i>)	Toxin A	Toxin B	CDT	Toxinotype
027	8	Pet food bowl (3), kitchen sink (2), kitchen sink tap, kitchen floor, toilet	None	Pos.	Pos.	Pos.	III
078	5	Fridge shelf (2), kitchen sink, kitchen sink tap, toilet	None	Pos.	Pos.	Neg.	V
L	5	Toilet (2), kitchen sink, kitchen counter, fridge shelf	Canine (1)	Pos.	Pos.	Neg.	0
001	5	Kitchen sink, fridge shelf, dog food bowl, toilet, fridge shelf	Canine (4), feline (2)	Pos.	Pos.	Neg.	0
Y	3	Fridge shelf, vacuum contents, kitchen counter	None	Pos.	Pos.	Pos.	III
V	2	Kitchen sink tap, dog eating area	Feline (1)	Pos.	Pos.	Neg.	0
AI	2	Kitchen counter, dog food bowl	Canine (2)	Pos.	Pos.	Neg.	0
C	1	Kitchen counter	Canine (1)	Pos.	Pos.	Pos.	IX
AA	1	Fridge shelf	None	Neg.	Pos.	Neg.	0
Q	0	None	Canine (2)	Pos.	Pos.	Neg.	0

exposure to an immunocompromised individual in the home remained significant when each of these potential confounders was included.

C. difficile was isolated from 44/836 (5.3%) sites in 26/84 (31%) households, ranging from 1 to 5 positive sites per household. The toilet was the most common site (9/83, 11%), followed by the dog food bowl, refrigerator shelf and kitchen sink (6/84, 7.1% each), kitchen counter, dog eating area floor and kitchen sink taps (4/84, 4.8% each), main entry and floor (2/84, 2.4% each) and vacuum bag contents (1/81, 1.2%). Only 4/26 (15%) households from which *C. difficile* was isolated from the environment had a pet positive for *C. difficile*. There was no association between the presence of a colonized animal in the house and detection of *C. difficile* in the household environment ($P=1.0$). Food bowls were 17 times (95% CI 1.02–300, $P=0.02$) as likely to be contaminated with *C. difficile* when a dog was fed a commercial raw food diet compared to those fed other types of diet. There was not a significant association between environmental *C. difficile* and any of the variables, including the presence of immunocompromised people in the home ($P=0.26$) or the ability of dogs to run freely in the park ($P=0.46$).

Sixteen of the 20 (80%) isolates from 13/17 (76%) animals were toxigenic, as were 32/44 (73%) environmental isolates (Table 1). All of the toxigenic strains have been previously isolated from humans in Ontario [9]. All four isolates from the dog with multiple positive samples were the same ribotype. In one household where *C. difficile* was isolated from a dog and a cat, both animals harboured the same strain (ribotype 001). In the other two households where more than one animal was colonized, the animals

carried different strains. Two or more different ribotypes were present in the environment in 8/44 (18%) households. *C. difficile* was isolated concurrently from dogs and the environment in four households, but in all cases canine and environmental ribotypes were different.

DISCUSSION

This is the first study evaluating *C. difficile* colonization in dogs and cats in a longitudinal manner, and results indicate that *C. difficile* is commonly albeit sporadically found in the faeces of healthy pets. Whether this represents intermittent true colonization, detection of ingested spores transiently passing through the intestinal tract or variation in results because of shedding near the detection threshold is unclear. While *C. difficile* colonization status is typically determined using single point-in-time sampling, this study suggests that such an approach may not be appropriate because of the inter-sample variation within animals. Whether point prevalence studies might overestimate (i.e. detection of transiently passing spores) or underestimate (i.e. intermittent shedding or limitation in detection threshold) is unclear, but these results indicate the need for further study to determine the dynamics of *C. difficile* shedding and relevance of results. Transient passage of spores should certainly not be dismissed given the presence of *C. difficile* in food [10, 11], water [11] and the household environment, and it cannot necessarily be assumed that a single positive sample truly represents colonization.

The most common ribotype found in animals was a strain that is commonly found in people. This

ribotype, 001, is a toxinotype 0 strain that was the most common ribotype found in hospitalized humans in Ontario in a recent study [9]. The other ribotypes found were less common in hospitalized people in Ontario but all toxigenic strains found in dogs and cats have been identified in people in Ontario. This is consistent with previous reports identifying commonality between human and animal *C. difficile* isolates [7, 8] and raises further concerns about whether interspecies transmission of *C. difficile* can or does occur. The nature of this study cannot answer those questions, yet it does provide additional circumstantial evidence suggesting that this may occur. Identification of the same ribotype in a dog and cat in the same household could represent direct interspecies transmission, indirect interspecies transmission through environmental contamination or infection of both from a common source.

The association between the presence of immunocompromised individuals in the household and *C. difficile* in pets has not been previously reported. The potential influence of human health status on *C. difficile* colonization in pets has been previously reported in a recent study that determined antimicrobial treatment of the pet owner was associated with increased *C. difficile* shedding by the dog [12]. For the current study, it is tempting to speculate that immunocompromised individuals are more likely to be colonized because of underlying disease or health-care system contact and subsequently infect their dogs; however, human samples were not obtained. This precludes making any objective assessment of humans as a possible source and this finding should be taken as an indication of the need for specific prospective study of *C. difficile* in immunocompromised people and their pets.

The presence of *C. difficile* in the household environment was rather common. This is, in some respects, surprising considering the assumption that *C. difficile* colonization is rare in healthy people in the general population, particularly when results of this study do not implicate pets as a significant source of contamination. However, there is limited contemporary information about *C. difficile* shedding in people in the community, particularly since the apparent dissemination of ribotypes 027 and 078 are lacking and it is possible that colonization rates are actually higher than has been assumed. Further, the spore-forming nature of *C. difficile* means that it can survive for prolonged periods of time once inoculated onto a household surface.

The only factor associated with contamination of any environmental surface was the association between feeding a commercial raw food diet and isolation of *C. difficile* from food bowls. It is probable that *C. difficile* contamination of raw meat, which has been previously reported in both retail meat [10, 13] and commercial raw pet foods [14] was the source.

Ribotype 027, the strain that was found most commonly in the environment, is a hypervirulent strain that has received much attention internationally, being associated with outbreaks and increased morbidity and mortality in North America and Europe [15, 16]. It is also common in endemic situations and was the second most common strain in a recent study of hospitalized individuals in Ontario [9]. The reason it predominated in the environment is unclear. There have been conflicting reports of the role of this strain in CA-CDI and little is known about colonization of healthy individuals in the community with ribotype 027. The relatively high rate of recovery of ribotype 078 from the environment was rather interesting in light of recent reports implicating it as a cause of CA-CDI [16, 17]. This ribotype is rare in hospitalized individuals in Ontario, accounting for only 1.8% of isolates in a recent study [9]. It has been most commonly associated with food animals and food, and concern has been raised about food as a source of infection [18]. Considering the number of kitchen sites that were positive and where ribotype 078 was found, it is possible that food was the source of contamination; however, this cannot be proven.

Although the prevalence of *C. difficile* in dogs and cats is low, the fact that all toxigenic strains are recognized human pathogens raises concern about interspecies transmission. The relationship between the presence of an immunocompromised individual in the home and *C. difficile* shedding by their dog supports this concern. The high prevalence of household contamination suggests that exposure to low levels of *C. difficile* may be a common event and the frequency of positive kitchen sites raises questions about food as the source of contamination. The relevance of *C. difficile* in both pets and the household environment requires further study to develop a broader understanding of the ecology and epidemiology of this pathogen in the community.

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DECLARATION OF INTEREST

None.

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