Clostroides difficile Infection: Interactions Between Humans and Dogs

Thesis

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By

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Abstract

C. difficile is a clinically significant healthcare associated infection, but recently there has been an emergence of a new class of *C. difficile* infection (CDI); community-associated (CA) – CDI. This presents added burden to the already significant issue. CDI is responsible for an estimated 453,000 cases and at least 29,000 deaths annually in the United States (Curry, 2017). As a spore former, it is difficult to remove from the hospital environment. *C. difficile* has been found in a wide variety of mammals, including dogs, and there is a potential for zoonotic transmission of *C. difficile* from dogs to humans. This study aims to determine if owning a dog or owning a dog that tests positive for *C. difficile* increases the odds of having *C. difficile.* This study will also examine the antibiotic resistance pattern of the *C. difficile* strains found in humans and dogs to see if they are the same. This study will accomplish this through a robust sampling and survey method. Patients at The Ohio State University Wexner Medical Center diagnosed with CDI were asked about whether they owned a dog and provided a fecal sample if they did. PCR ribotyping and antibiotic resistance typing is used to examine the genetic relatedness of strains isolated from humans and their dogs and indistinguishable strains could represent transmission. This study provided data to support that *C. difficile* was not transmitted between dogs and humans.

Keywords: *C. difficile;* dog; PCR ribotyping; antibiotic resistance

Dedication

This thesis is dedicated to my dog Bear, who to the best of my knowledge does not have *C. difficile.*

Acknowledgments

I would like to thank Dr. Jason Stull for putting me in contact with this dataset and always answering my questions. I would also like to thank Dr. Thomas Wittum and Dr. Gregory Habing for all the work they put in collecting the data and allowing me to work on this dataset. Finally I'd like to thank Dr. Dixie Mollenkopf for the laboratory results and guidance in understanding them.

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Fields of Study

MAJOR FIELD: Public Health

Table of Contents

Abstract ii
Dedication iii
Acknowledgments iv
Vita v
List of Tables vii
Chapter 1. Introduction 1
Chapter 2. Background……3
Chapter 3. Methods……14
Chapter 4. Results……..17
Chapter 5. Discussion……....21
Bibliography 23

List of Tables

Table 1. Results of C. difficile tests by whether a dog was in the study……..……17

Table 2. Results of C. difficile testing for dogs in the study and their owners……..18

Table 3. Matching antibiotic resistance patterns for C. difficile and other enterobacteria species……....18

Table 4. Results of univariate logistic regression for each variable……..19

Chapter 1. Introduction

C. difficile can be found in the feces of most mammals, including dogs (Curry, 2017). The close relationship many people have with their companion dogs provides a possible route of infection. Many people allow their dogs to lick their faces without a second thought or share a bed with their dogs (Amiot, 2016). This presents a convenient route for zoonotic infections. Dog owners often consider their dogs family members and are extremely close with them. Dogs can carry many diseases that are harmful to humans. Fortunately, we vaccinate for the worst offenders. Unfortunately, there is no vaccine for *C. difficile.* The spores of *C. difficile* are incredibly hardy and once tracked into the house by a pet can remain infectious indefinitely (Curry, 2017).

The epidemiology of *C. difficile* changed drastically at the turn of the 21st century, with an increase in the number of cases, the severity of cases, and the number of deaths (Curry, 2017). From 1993 to 2003 *C. difficile* incidence doubled in the United States and a new strain, rarely seen before, accounted for 30% to 50% of cases (Curry, 2017). Another troubling development in the epidemiology of *C. difficile* occurred in 2005 when severe CDI was reported in low risk patients without exposure to antibiotics (Curry, 2017). Traditionally considered primarily a healthcare associated (HA) infection, recent cases of *Clostroides difficile* infection (CDI) have presented without any of the normal risk factors. This has led to a new designation, community-associated (CA) CDI (Cohen,

2010). How humans come to be infected with *C. difficile* from the environment is complicated by the ubiquitous distribution of the bacteria (Otten, 2010). One possible route of transmission is from companion animals. The prevalence of CA – CDI is much lower than the prevalence of HA – CDI (Curry, 2017). Despite this, the reproduction number of within-hospital transmission is too low to explain all cases of *C. difficile* (McLure, 2017). Therefore; CA – CDI must play a role in the total number of cases and controlling it will help control HA – CDI.

This study will examine the relationship between testing positive for *C. difficile* and having a dog also test positive for *C. difficile.* Patients at The Ohio State University Wexner Medical Center (OSUWMC) that tested positive for *C. difficile* were contacted about participating and if they agreed were given a survey and a dog feces sample collection kit. The survey included important clinical information about the patient and their dogs. When returned, the dog samples were also tested for *C. difficile.* It was expected that having a dog test positive for *C. difficile* would put the patients at greater risk of also having *C. difficile.* This could indicate that there was transmission between dogs and humans.

This study aimed to show that individuals who own dogs are at higher odds of CDI than those who do not own dogs and that owning a dog that tested positive for *C. difficile* will also increase the odds of CDI. Additionally, antibiotic resistance patterns were examined to determine if having a matching pattern between human and dog could indicate transmission and a higher risk of CDI.

Chapter 2. Background

C. difficile is a clinically important anaerobic spore forming bacteria found in the intestinal tract of humans and animals (Curry, 2017). The bacteria are also very prevalent in soil (Paediatr Child Health, 2000). *C. difficile* is a gram-positive bacillus and resistant to many standard sterilization procedures in its spore form (Heinlan, 2011). In the gut of a patient receiving antibiotic therapy, *C. difficile* can convert to its disease-causing form (Bella, 2016). This is initiated by the presence of glycine and cholate derivatives that are usually degraded by other healthy gut bacteria (Bella, 2016). The disruption of healthy intestinal microflora allows for *C. difficile* overgrowth and clinical disease.

C. difficile produces two toxins, A and B, both of which can cause severe disease (Carroll, 2011). Toxin A and B are members of the large clostridial glycosylating family (Bella, 2016). The toxins bind to cells and then are taken into the cell through receptor mediated endocytosis (Bella, 2016). Next the toxins translocate from the endosome into the cytosol (Genisyuerek, 2011). Exactly how the toxins do this is unclear. Barth et al. demonstrated that toxin B changes shape at low pH inside the endosome which allows it to insert into the membrane and form a channel (2001). Geisemann et al. showed that both toxin A and B are inserted into the membrane, form a pore and release the catalytic region into the cytosol in a way that is dependent on the presence of cholesterol (2006). Once in the cytosol the toxins begin glycosylating several Rho subfamily proteins, causing inactivation (Bella, 2016). These proteins have many functions including cell cycle progression, cytoskeletal regulation, cell division, phagocytosis regulation, and cytokine production (Bella, 2016). The disruption of the cytoskeletal structure of cells

causes malformation and death (Bella, 2016). The genes for the two toxins are located on the Pathogenicity locus (PaLoc) along with negative and positive regulators of the toxin genes (Bella, 2016). Strains of *C. difficile* that lack the PaLoc are nontoxigenic (Curry, 2017).

C. difficile has additional virulence factors that are outside the PaLoc such as the binary toxin (Curry, 2017). This toxin is encoded by two genes and is common in the epidemic 027 strain, although the contribution of this toxin to the overall toxicity of *C. difficile* is not understood (Curry, 2017). Passmore et al. demonstrated that *C. difficile* produces para-cresol which inhibits the growth of normal gram-negative intestinal bacteria providing *C. difficile* a competitive edge (2018). *C. difficile* also has a number of proteins on its S-layer that facilitate adhesion to and invasion of tissues (Vendantam, 2012).

Identified in 1935 in the feces of healthy infants, it was not known *C. difficile* caused disease until 1977 when it was shown to be responsible for what was known as "antibiotic-associated colitis" (Curry, 2017). *C. difficile* was demonstrated to be the organism responsible for human disease and the majority of antibiotic-associated diarrhea in 1978 (Heinlen, 2011). *C. difficile* is transmitted via the fecal – oral route (McDonald, 2019). *C. difficile* infection only occurs when colonized individuals demonstrate clinical symptoms. Typically, two events need to occur for CDI to present with pathological consequences; disturbed fecal microbiota and ingestion of *C. difficile* spores (McDonald, 2019). Once ingested the spores germinate in the large intestine in response to the presence of bile salts (Sorg, 2008). This ensures that the spores are in the proper location

before germinating. *C. difficile* then colonizes the large intestine. Colonization of other areas of the body, including the small intestine is rare, owing to the anaerobic environment and lack of competitive bacteria in the large intestine (Heinlen, 2010). Adhesion is facilitated by microtubule extensions of the intestinal epithelium caused by the toxins produced by *C. difficile* (Heinlen, 2010). The toxins cause conformational changes, fluid secretion, inflammation, and necrosis (Heinlen, 2010).

The loss of normal intestinal microflora and infection with *C. difficile* is a disastrous combination. The inflammation and death of intestinal epithelial cells compromises the body's ability to absorb water and nutrients, leading to the diarrhea associated with CDI. The clinical presentation of CDI can range from mild diarrhea to fulminant colitis, otherwise known as pseudomembranous colitis (Curry, 2017). In some cases CDI can be fatal. Before 2001, the most common presentation of CDI was mild diarrhea (Curry, 2017). Leukocytosis is also common in CDI and has CDI been found to be the most common cause of unexplained inpatient leukocytosis (Curry, 2017). CDI can also cause the passage of blood and mucus in the stool (Cohen, 2010). Fever is observed in less than 50% of patients (Curry, 2017). Since CDI begins with mild diarrhea it is sometimes misdiagnosed or missed entirely (Heinlen, 2010). This is problematic as the disease can progress to a much more serious issue very quickly.

Pseudomembranous colitis (PMC) is a severe complication of CDI. *C. difficile* is the most common cause of PMC but other causes are possible and before wide spread use of broad-spectrum antibiotics ischemic disease, obstruction, and sepsis were the most frequent (Farooq, 2015). PMC presents as yellow to yellow-white plaques that appear on

the mucosal surface of the intestinal epithelium (Kawamoto, 1999). These plaques form in response to the necrosis caused by CDI (Farooq, 2015). The body's immune response floods the affected area with neutrophils and other inflammatory elements, while dead cells and bacteria build up forming a sort of pseudo-membrane of debris, hence the name (Farooq, 2015). If severe disease continues, pseudomembranous can cover the entire intestinal mucosa (Carpenter, 2000). PMC can lead to distension of the colon, known as toxic mega colon (Heinlen, 2011). Progression from mild disease to PMC varies from patient to patient and can occur as quickly as a few hours or as long as a few weeks after initial infection (Heinlen, 2011). PMC is a life-threatening condition and a significant cause of morbidity and mortality associated with CDI (Dallal, 2002). Surgical treatment of PMC carries with it a high risk of death (Dallal, 2002).

The classical risk factors for CDI are well understood, the most important amongst them is recent antibiotic treatment. Clindamycin and cephalosporins are widely implicated in CDI having the highest incidence and prevalence, respectively (Carroll, 2011). Arronson et al. demonstrated that the relative risk of CDI associated with clindamycin and cephalosporins was 10 to 70 times greater than other antibiotics (1985). Olson et al. showed that 96% of CDI cases had received antibiotics within the last 14 days and all had received an antibiotic in the last month (1994). Fluoroquinolones have become a serious inducing agent since their widespread use starting in the 2000s and have been implicated in severe outbreaks that were only stopped by restricting the use of fluoroquinolones (Carroll, 2011). Advanced age and length of stay in a hospital are also risk factors for CDI. There is an extreme increase in incidence in people over 65 and

there is a direct correlation of risk and age above 65 years (Arronson, 1985). Contact with the healthcare system in any way is a risk for CDI and the longer the stay the higher risk (Carroll, 2011). The hospital environment is heavily contaminated with *C. difficile* from surfaces and hospital personnel to asymptomatic carriers and even the air (Carroll, 2011).

Asymptomatic carriers of *C. difficile* represent an important route of infection. Patients visiting hospitals for other issues can track the bacteria and its spores in, leading to many HA infections. Many studies have shown that 50% of patients with *C. difficile* are asymptomatic carriers (Cohen, 2010). *C. difficile* is widespread in the environment and can be found in the intestines without clinical disease (Caroll, 2011). About 5% of the general population is colonized with *C. difficile* but remain protected from symptomatic disease by healthy intestinal flora (McLure, 2017). One prospective study even showed that up to 62% of patients who acquired *C. difficile* from a hospital were asymptomatic (Curry, 2017). Asymptomatic carriers must play a critical role in the spread of CDI as mathematical models have shown that symptomatic CDI patients cannot account for all transmission events (Curry, 2017). After resolution of symptoms individuals can remain colonized for weeks or even longer, contaminating their environment and others in it (Sethi, 2010). Studies have suggested that CDI prevalence is greater than previously estimated and with the threat of antibiotic resistance, more virulent strains are becoming common (Jarvis, 2008).

The burden of *C. difficile* on the healthcare system is immense. In 2013 there were 250,000 CDI – related hospitalizations and at least 14,000 deaths (CDC, 2013). In 2017, Curry describes over 453,000 cases and 29,000 deaths annually in the United States

(2017). *C. difficile* has replaced *Staphylococcus aureus* as the most common HA infection (Curry, 2017). A 2008 study examined the nationwide prevalence of CDI and found 1,443 *C. difficile* positive patients out of 110,550 patients total for a prevalence of 13.1 per 1,000 inpatients (Jarvis, 2009). 94.4% of these were active infection and 5.6% were asymptomatic carriage (Jarvis, 2009). The lowest prevalence rate was observed in Hawaii; 0 cases, and the highest was observed in Rhode Island; 28.9 cases per 1,000 (Jarvis, 2009). The average cost to treat CDI was $42,316 and the average attributable cost to CDI was $21,448 in 2015 (Zhang, 2016). HA – CDI was 1.5 times as expensive as CA – CDI. (Zhang, 2016). Many patients experience recurrence of CDI after resolution of symptoms and return to the hospital, adding to costs (Zhang, 2016). In 2015, in the United States, the total attributable cost of CDI was 6.3 billion dollars (Zhang, 2016).

A big change occurred in the epidemiology of *C. difficile* in 2000. What was before a concern when using antibiotics, was now an absolute nightmare. Disease incidence and severity skyrocketed starting in 2000 (Curry, 2017). The number of deaths, cases of PMC, and cases resulting in colectomy began increasing (Curry, 2017). CDI doubled in the United States from 1996 to 2003 (McDonald, 2006). A new strain, rarely seen before 2000, developed increased resistance to fluoroquinolones, and went on to become one of the most common strains of *C. difficile* (McDonald, 2005). This strain is named NAP1 by pulse field gel electrophoresis, 027 by polymerase chain reaction ribotyping and ST1 by multi locus tandem repeats genotyping (Curry, 2017) It is commonly referred to as NAP1/B1/027. Although this strain has become widespread the current literature is undecided as to the causal relationship between its emergence and the

increase in severity of CDI observed since 2000 (Curry, 2017). McDonald et al. demonstrated that the increase in severity could not be attributed to this new strain alone and concluded that host susceptibility, current practices, and the use of antimicrobials could be to blame (2005).

The next big change in CDI epidemiology occurred in 2005 when populations without the classic risk factors began to become ill with CDI. The CDC describes multiple cases of CDI in healthy individuals with no exposure to the healthcare setting and pregnant women; populations that were traditionally at low risk for CDI (2005). A surveillance study in 2006 conducted by the CDC found the incidence of CA – CDI to be 6.9 cases per 100,000 (2006). Lessa et al. conducted a surveillance study across the united states and estimated the national incidence of CA – CDI to be 51.9 per 100,000 for a total case burden of 159,700 (2015). In this study, CDI was defined as community-associated if a positive specimen was collected from an outpatient setting or less than 3 days after admission to a hospital with no previous stay in a hospital within the last 12 weeks (Lessa, 2015). This is significantly greater than the estimates provided by the CDC in 2006. CA-CDI is closely related to HA-CDI. McLure et al. described a mathematical model of *C. difficile* in Quebec where the reproductive number was below 1, which shows that HA-CDI alone could not account for all the disease found (2017). The epidemiology of *C. difficile* continues to evolve and staying on top of this evolution is crucial to combating the disease.

It is important to understand where CA-CDI is coming from. There are currently four broad categories in which CA-CDI can be separated into; consumption, person-to-

person, animal-to-person and environmental (Otten, 2009). Infection can occur in the community from consumption of contaminated food or drink (Otten, 2009). Studies have shown that tap water and vegetables can sometimes be contaminated with *C. difficile* spores (Otten, 2009). Samples of ground meat collected from Ontario and Quebec stores were found to be *C. difficile* positive 20% of the time (Rodriguez-Palacious, 2007). Although foodborne CDI is plausible, prevalence studies have shown prevalence is low and even in the rare cases where there is contamination, the colony counts are very low (Curry, 2017). Direct contact with infected family members with active disease may be a significant risk factor for infection as individuals with diarrheal symptoms shed more *C. difficile* than healthy colonized individuals (Otten, 2009). Symptomatic persons shed large numbers of spores in their stool which contaminates themselves, their clothes, and their environment, creating what has been described as a "fecal veneer" (Donskey, 2010). Contact with infected or colonized animals can be a source of infection. CDI is documented in many animals, but proof of transmission does not exist (Otten, 2009). CDI rates in dogs have been described as between 0 to 26% and 2% to 32% in cats (Otten, 2009). For both cats and dogs, veterinary inpatients have higher rates of CDI, like humans (Otten, 2009). Arroyo et al. conducted a PCR ribotyping study of *C. difficile* in humans, dogs, horses and one cat and one calf (2004). They showed that 25% (5/20) of isolates were indistinguishable between humans and at least one animal species, supporting the possibility of animal to human transmission (Arroyo, 2004). People can become infected directly from the environment as well. As stated earlier, *C. difficile* is widespread in the environment and provides numerous routes of infection. In addition to

the natural range of *C. difficile*, infected or colonized individuals can contaminate the home or community environment (Otten, 2009).

Although *C. difficile* colonization is prevalent in the dog population, little is known about the role it plays in canine disease. *C. difficile* organisms have been isolated from clinically ill and asymptomatic dogs (Weese, 2010). Studies have shown *C. difficile* is associated with 10% to 21% of cases of diarrhea in dogs but causation has not been proven (Marks, 2011). Administering *C. difficile* to healthy dogs without antibiotics did not cause disease (Marks, 2011). Despite this *C. difficile* is quite prevalent in dogs. In one study of a veterinary setting 19% of dogs tested were positive for *C. difficile*, and of these 69% were toxigenic strains (Clooten, 2007). Weese et al. described an outbreak involving 93 dogs and causing one death in a single veterinary hospital (Weese, 2003). Some studies have found that up to 58% of healthy dogs and cats are colonized with *C. difficile* (Marks, 2011). *C. difficile* strains isolated from dogs and cats are sometimes indistinguishable from human isolates (Weese, 2009). Toxigenic strains of *C. difficile* have been isolated in the feces of both healthy dogs and dogs with diarrhea (Wetterwik, 2013). Weese et al. conducted a study of *C. difficile* strains in Quebec and found the most commonly found ribotype in animals was one that is common in humans (2009). This could point to possible transmission from dogs.

Risk factors for *C. difficile* colonization in dogs is not as well understood as in humans but share some similarity. An immunocompromised owner, antibiotic treatment of the dog, antibiotic treatment of the owner, contact with children, and contact with the healthcare setting are all risk factors for dogs (Marks, 2011). Prevalence of colonization

in dogs is high but the incidence of clinical disease is low suggesting that infection is dependent on some other factors having to do with the interaction of the host, the host's immune system, and *C. difficile* (Marks, 2011). The estimated prevalence of *C. difficile* in the dog population is between 5-10%, with up to 40% in veterinary inpatients (Weese, 2008). Dogs that visit human hospitals as a part of animal – assisted interventions are at higher risk of being colonized with *C. difficile* (Lefebvre, 2009).

Colonized dogs shed *C. difficile* spores in their feces and without clinical disease it is not possible to know if a dog is colonized. This presents a very viable route of transmission from dogs to human. As stated earlier, the *C. difficile* spores shed in feces can coat many surfaces and once brought into a home by a dog there can be widespread contamination. Janezic et al. examined *C. difficile* contamination in the household and found the highest positivity rate and variability in ribotype on shoes, slippers, and dog paws (2018). Dog paws could represent a significant source of household contamination and CA-CDI (Janezic, 2018). The high variability of ribotypes shows demonstrates that dogs are collecting many strains from the environment and then bringing them into the home.

Given the clinical significance of CDI, the prevalence of colonization in dogs, and the same strains being found in humans as in dogs, understanding the role dogs play as possible vectors of infection is extremely important. Although a zoonotic route has not been identified, "circumstantial evidence points to a zoonotic potential" (Hensgens, 2012). According to the literature the role of *C. difficile* as a zoonotic disease of humans is not well characterized. By sampling dog owners and their dogs and using polymerase

chain reaction (PCR) and antibiotic resistance screening this study aims to describe the transmission of *C. difficile* from dogs to humans. To the best of knowledge this study represents the first to examine the role of dog colonization with *C. difficile* on human infection with *C. difficile* by targeting humans with and without *C. difficile* who owned and did not own dogs.

Chapter 3. Methods

A case control study design was used. Cases were defined as testing positive for *C. difficile* and controls were defined as testing negative for *C. difficile.* Owning a dog was the main exposure of interest. Owning a dog that tested positive for *C. difficile* was also examined. At the Ohio State University Wexner Medical Center (OSUWMC) human CDI testing was performed using Xpert C. difficile/Epi. This test is a PCR gene assay that detects the presence of Toxin B DNA. This test is extremely sensitive as any amount of Toxin B DNA will be greatly amplified by PCR (Pancholi, 2012). A daily list of patients undergoing *C. difficile* testing was provided to the research staff via OSUWMC email, a secure system. If the patient was non-critical, the primary physician was contacted and requested to ask their patient if they would like to participate in the study. If the patient agreed, the investigator approached the patient to provide additional study details and answer any questions.

The inclusion criteria for the study was having a *C. difficile* test ordered. Patients that met the inclusion criteria for the study were consented. Consent was obtained by having participants read a consent form. The form detailed their involvement in the study and permission for OSUWMC to provide the investigator with information about the participants *C. difficile* test, specifically the date and results. A study member was available for any questions and the participants gave verbal consent. After consent participants were provided with a study kit. For dog owners, the study kit included a description of the study, a consent form, a survey, a $5 incentive, a collection kit for dog feces, and a postage-paid return envelope. The survey was 5 or 15 minutes long

depending on whether the participant owns a dog. It was requested that participants collect two stool samples from up to two household dogs. Dog owners were given the opportunity to participate in a follow up to this study by providing an additional dog fecal sample exactly one month after the return of the original kit. The same monetary incentive was provided.

The OSUWMC stores patient stool samples for 7 days in case additional testing is ordered. Once a patient consents, instead of being discarded, the sample got a unique study ID number for deidentification, and sent to the OSU College of Veterinary Medicine for *C. difficile* culture and additional testing. The questionnaires participants received were developed with an interdisciplinary team of epidemiologists, veterinarians, and infectious disease physicians. The sampling kits contained instructions for collection and all the necessary materials. Two fresh samples were requested because intermittent shedding has been observed in healthy dogs (Weese, 2010). Each dog stool sample was cultured and tested for *C. difficile*. When participants consented their stool samples were also cultured. Dogs were considered positive for *C. difficile* if one or more of the samples were positive. Cultured *C. difficile* was tested for antibiotic resistance against Cefepime and Extended-Spectrum β-Lactamase phenotype, Meropenem and Carbapenem, and Ciprofloxacin and Fluoroquinolone. A unique identification system was used for all surveys and samples. The investigators on the study never had access to the medical record numbers of the patients. Survey data was entered into a database without any identifying information except for a unique ID number. All data was stored on secure servers stored at the College of Veterinary Medicine and OSUWMC. Access is restricted

and only study staff had access to the data. The paper surveys were stored in a locked cabinet and retained for 5 years. At the conclusion of the study the identifiers were destroyed. The identifiers linking the surveys and the sample data were also destroyed. The OSUWMC destroyed the log linking the unique ID with patient medical record numbers.

The results of the survey about human and dog health were combined into a dataset along with the results of the *C. difficile* tests. 154 human samples were tested for *C. difficile*. Of these, 31 had dogs. 3 samples could not be associated with the related dogs and were dropped from the analysis leaving 28 participants with dogs in the study. Fischer's exact test was used to determine if having a dog in the study was a predictor of CDI. The results of the survey on dog health were available for 28 dogs. These results were associated with the human samples to determine if any specific dog health outcomes were significantly associated with CDI in humans. Fischer's exact test was used.

Antibiotic resistance screening was conducted on 88 human samples and 90 canine samples. The results were examined to see if human isolates had resistance patterns that matched at least one of their dogs. Fischer's exact test was performed to see if a significantly greater proportion of *C. difficile* isolates matched than other enterobacteria encountered. Logistic regression was used to determine if any of the data collected in the survey was a significant predictor of CDI. A model was created using purposeful selection of covariates, described by Hosmer and Lemeshow (2013). Stata 15 was used for all statistical analysis.

Chapter 4. Results

	C. difficile Positive			
Dogs in Study	Yes	No	Total	Odds
Yes	10 (6.49%)	18 (11.69%)	28 (18.18%)	0.56
No	33 (21.43%)	93 (60.39%)	126 (81.82%)	0.35
Total	43 (27.92%)	111 (72.08%)	154 (100.0%)	0.39

Table 1. Results of *C. difficile* tests by whether a dog was in the study.

The results of the *C. difficile* testing by whether the person also had a dog in the study is shown in Table 1. 43(27.92%) human samples tested positive for *C. difficile*. People who had dogs in the study had 1.57 the odds of testing positive for *C. difficile* than people who did not have dogs in the study. (95% CI = 0.58 – 4.01) The most common toxin profile encountered was Toxin A (A) positive Toxin B (B) positive Binary Toxin (CDT) negative. 12 (8.9%) dog samples tested positive for *C. difficile*. The toxin profile was 6 A+B+CDT- and 6 A-B-CDT-. 2 of these samples could not be associated with their humans. None of the human samples with dogs in the study had the same toxin profile as their dogs. There was no significant association between having a dog in the study and testing positive for *C. difficile*.

	C. difficile Positive			
Dog *C. difficile* Positive	Yes	No	Total	Odds
Yes	1 (3.57%)	6 (24.43%)	7 (25.0%)	0.17
No	9 (32.14%)	12 (42.86%)	21 (75.0%)	0.75
Total	10 (35.71%)	18 (64.29%)	28 (100.0%)	0.36

Table 2. Results of *C. difficile* testing for dogs in the study and their owners.

Next dogs that tested positive for *C. difficile* were compared with their owners. 10 (35.71%) dog owners tested positive for *C. difficile* while 18 (64.29%) were negative. People who owned a dog that tested positive for *C. difficile* had 0.22 times the odds of having *C. difficile* as those who did not have a positive dog (95% CI = 0.043 – 2.49). There was no significant association between having a dog test positive for *C. difficile* and the owner testing positive for *C. difficile*.

	C. difficile Positive			
Resistance Pattern Match	Yes	No	Total	Odds
Yes	5 (22.73%)	7 (31.82%)	12 (54.55%)	0.71
No	2 (9.09%)	8 (36.36%)	10 (45.45%)	0.25
Total	7 (31.82%)	15 (68.18%)	22 (100.0%)	0.47

Table 3. Matching antibiotic resistance patterns for *C. difficile* and other enterobacteria species.

12 of the human samples had matching antibiotic resistance to the samples of their dogs in the study. The risk ratio of *C. difficile* comparing matching antibiotic resistance pattern to not matching was 2.08 (95% CI = 0.51 – 8.52). There was no

significant association between matching resistance patterns and testing positive for *C. difficile*.

Variable	Odds Ratio	P-value	95% CI
Dog *C.difficile* Positve	0.22	0.15	0.023 – 2.18
Wash Hands Before	2.93	0.096	0.78 – 11.05
Wash Hands After	4.70	0.0073	1.29 – 17.11
Dog Contact With Other Animals Y/N	0.29	0.25	0.029 – 2.91
Dog on Antibiotics Y/N	1.89	0.67	0.11 – 33.89
Age (Human)	1.008	0.72	0.96 – 1.06
Gender (Human)	2.40	0.32	0.72 – 13.6
Chronic Illness Y/N (Human)	0.51	0.41	0.10 – 2.57
Antibiotic Y/N (Human)	0.55	0.42	0.11 – 2.67
Acid Suppressant Drug Y/N (Human)	0.46	0.39	0.07 – 2.89
Immune Suppressant Drug Y/N (Human)	0.89	0.88	0.19 – 4.24
Hospitalized in last month Y/N (Human)	0.23	0.25	0.02 – 3.03
Diarrhea Y/N (Human)	0.32	0.21	0.054 – 1.90
Diagnosed with *C. difficile* before (Y/N)	1.6	0.57	0.31 – 8.25

Table 4. Results of univariate logistic regression for each variable.

For the model, first a univariate logistic regression for each variable collected from the survey and the testing results was conducted. Having a dog test positive for *C. difficile*¸ whether or not hands were washed before, and whether or now washed after were significant at the p=.2 level. Whether or not the person was experiencing diarrhea had a p-value of .2061, and since diarrhea could be an indicator it was included in the initial model. This gave a model including whether an owner's dog tested positive for *C. difficile*, washing hands before, washing hands after, and diarrhea status. Washing before

and washing after was coded using 2 dummy variables each to describe sometimes washing, and always washing, with a reference of never/rarely washing. There were no observations of always washing before handling the dogs and it was omitted. None of these variables were significant at the p=.05 value. Variables were dropped one at a time starting with the largest p-value. At no point did any of the variables become significant. Variables that were originally excluded were entered into the model, including age, contact with the healthcare system, and chronic illness status, as the literature shows these to be predictors of CDI. Still no variables were significant predictors of testing positive for *C. difficile*.

Chapter 5. Discussion

The literature clearly describes CDI as a significant health burden in the United States. The development of antibiotic resistant and community associated strains is a very concerning turn for this once only health care associated infection. Limiting *C. difficile* infections from the environment will hopefully lead to a reduction of hospital associated infections as people will not be bringing these infections with them. Determining the routes of infection from the environment is crucial for stopping the infection. Better preventative and educational interventions can be disseminated when the exact mechanism of infection is known. This study provides evidence to the growing body of research that concludes dogs are not a source of CA-CDI

Based on the results of the survey the participants in this study had very close contact with their dogs. Many described being licked on the face by their dogs or not washing their hands when playing with them. The dogs were also allowed outside and, in some instances, had contact with other animals. Despite this no households had a human sample and a dog sample that had matching toxin profiles. This provides evidence that no transmission events occurred.

The antibiotic resistance patterns of the enterobacteria strains encountered in this study did match between human and dog samples in some cases. This does not imply that transmission occurred and was not significantly different for *C. difficile* compared to other bacteria. Antibiotic resistance genes are becoming widespread and bacteria can

share these genes. It is just as likely that these strains had similar antibiotic resistance patterns by chance or that a common exposure route exists.

A major strength of this study was the case control methodology and the large number of patients with *C. difficile* recruited. The *C. difficile* test used was very specific. The survey was well developed and contained all the important information required. The major limitation of this study was the small sample size for participants with dogs. Although CDI rates are high and many samples were tested, the number of people that included their dogs in this study was low and this brings into question the significance of the results. Another issue is that for people who did not enroll their dogs in this study, we do not know that they do not have dogs, we only know they were not enrolled in the study. Additionally, of those that completed the survey many did not answer all the questions. There was a significant partial non-response bias. Only non-critical patients were included in the study, excluding a subset of the population of infected. Finally, although indistinguishable strains can indicate transmission, another possible explanation is a common source of exposure.

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