

# *Clostridium difficile* as a Risk Associated with Animal Sources

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## Introduction

*Clostridium difficile* was first described as part of the normal microbiota in stool samples from healthy infants in 1935 (59) and is still detected in significant numbers of healthy asymptomatic infants (143). Later, it was identified as a pathogen associated with pseudomembranous colitis and occasionally with wound and lung infections. (12;96;153;153) Now it has become the most common cause of diarrhea in hospitals and long term care facilities causing billions of dollars in excess costs. (43) *C. difficile* contributes to the death of an estimated 14,000 people annually in the U.S. and over 90% of the fatalities are >65 years of age. (105) The elderly and those being treated with antibiotics to control other infections are most susceptible to *C. difficile*. Broad spectrum antibiotics destroy much of the normal intestinal microbiota allowing some resistant bacteria (such as *C. difficile*), that are normally not very competitive in this environment, to thrive.

Although most cases of *C. difficile* infection (CDI) occur in patients in health care facilities, there has been a recent increase in community-acquired infections. *C. difficile* spores have been detected in meat, seafood, and some vegetables indicating a potential for foodborne transmission. *C. botulinum* and *C. perfringens* have been food safety concerns for decades because they produce potent toxins and their spores survive desiccation, many thermal treatments, and other preservation methods. Other clostridial species are known spoilage organisms. As yet, there has been no definitive proof that humans acquire *C. difficile* from contaminated food. However, since *C. difficile* is present in livestock and its spores survive ordinary cooking temperatures and some food processing conditions, foodborne transmission should be considered a possibility. This white paper will provide an overview of the association between *C. difficile* and human disease and summarize currently available information from the scientific literature and government reports on the presence of *C. difficile* in foods and in food-producing and companion animals. Epidemiology and characteristics of toxigenic strains associated with community and hospital associated outbreaks will be described.

## *Clostridium difficile* – biology and pathogenesis

### Biology

As with other clostridia, *C. difficile* is a Gram positive, spore-forming, obligate anaerobe. It grows more slowly than other clostridia and this makes it more difficult to isolate because it is often overgrown by other bacteria in mixed cultures. Sequencing of the genome of a virulent epidemic strain revealed that *C. difficile* shares only about 15% of its coding sequences with *C. botulinum* and *C. tetani*. About 11% of its genome consists of mobile genetic elements, some of which contain antibiotic-resistance genes. These mobile elements include transposons and prophages which can be passed horizontally from one *C. difficile* cell to another and have likely played an important role in the rapid evolution of *C. difficile* in the past decade. Some prophages are induced during infection and can be isolated as free viral particles from fecal samples. (108)

*C. difficile* has numerous adaptations allowing it to grow and survive in the mammalian intestine including the ability to tolerate bile acids and to degrade ethanolamine, an important compound providing carbon and nitrogen for growth. *C. difficile* can synthesize and tolerate high levels of a bacteriostatic compound, *p*-cresol (which smells like horse manure). This compound

may enhance its competitiveness against other intestinal microbes. (27) A dog has been trained to detect the odor of *C. difficile* and can identify patients with CDI. (21)

### **Virulence**

*C. difficile* produces two major toxins, TcdA and TcdB, that affect normal physiological reactions in target intestinal cells resulting in colitis and *C. difficile* associated diarrhea (CDAD). (177) These toxins are related to other large clostridial toxins that are produced by *C. novyi* and *C. sordelii*. The toxins inactivate small GTPase enzymes in cells and are synthesized primarily during late log and early stationary phases of growth. Some data demonstrate that TcdA levels correlate well with disease symptoms and antibodies against TcdA protect against disease. The role of TcdB has not been as well delineated. Another toxin, CDT, which is a binary toxin, is produced by some *C. difficile* strains but its role in pathogenesis is not well understood. In one study involving 265 patients, those infected with strains producing the binary toxin had a higher case-fatality rate than those infected with strains not producing this toxin. (7) Further details on the actions of these toxins were discussed in these reviews. (144;177)

A hypervirulent, epidemic strain of *C. difficile* emerged in the early 2000s in North America, causing severe illness with high fatality rates. It belongs to PCR ribotype O27, North American pulsotype 1 (NAP1), and is of toxinotype III. This strain is resistant to fluoroquinolones, produces the binary toxin, has an 18 bp deletion in the toxin regulator gene (*tdcC*), and produces higher levels of toxins and spores than other strains. (46;104;118;187) The *tdcC* gene is thought to be a negative regulator of toxin production although evidence indicates that the regulatory system is complex involving several factors. (29)

By 2005, this strain had spread to Europe, also causing large, severe outbreaks of CDI. (31) Several large, multi-hospital outbreaks occurred in the U.S. (81) and Europe (2;19) Then, in 2012, there were reports of O27 isolates from Latin America. (67;129) Ribotype O27 continues to evolve with some new strains displaying novel characteristics. (171)

Genome analyses of 151 strains of BI/NAP1/O27 isolated primarily from hospital patients (1994-2010) have revealed the evolution of two distinct lineages of the hypervirulent epidemic strain. One lineage apparently originated in northeastern US with the earliest isolate detected in Pittsburgh in 2001 and the other also originated in North America (Canada or the U.S.) and was first associated with an outbreak in Montreal in 2003. The two consistent genetic differences between these epidemic strains and related non-epidemic precursor O27 strains were the acquisition of a mutation encoding fluoroquinolone resistance and of a transposon (mobile genetic element) containing genes for DNA-binding protein(s), regulators of RNA synthesis, and a transport system. These changes apparently improved the fitness of these strains allowing them to spread rapidly throughout North America and to Europe and Australia. (64)

Another hypervirulent strain, O78, was described in 2008 in Europe as the cause of severe infections. This strain has similar virulence characteristics to the O27 strain but its deletion in the toxin regulator gene is larger. O78 appears to cause more community associated cases of CDI and affects a younger population than O27. This strain is similar to some *C. difficile* isolates from swine. (55) Strain O78 has also spread internationally.

Several other hypervirulent *C. difficile* isolates with increased sporulation and toxin production have been described. (109) DNA markers for *C. difficile* strains associated with severe disease were identified after a comparative genome analysis of 14 isolates. (48)

### **Antibiotic Resistance**

Early clinical studies of *C. difficile* in the 1970s indicated these bacteria were resistant to some antibiotics, most notably, clindamycin. Later, cephalosporin use became a prominent risk factor for CDI and more recently, important *C. difficile* strains have exhibited resistance to fluoroquinolones. (11) Examination of the genomes of many *C. difficile* isolates has demonstrated the presence of a wide range of mobile elements encoding resistance to many antibiotics including erythromycin, chloramphenicol, tetracycline, and aminoglycosides. Fluoroquinolones were one of the most commonly prescribed classes of antibiotics in the late 1990s in North America and this apparently exerted the selective pressure for the evolution of the virulent 027 ribotype strains in hospital settings. (64)

Many strains have acquired multiple transposons encoding resistance to different antibiotics and are now classified as multi-resistant strains. Examination of 316 *C. difficile* isolates from North America indicated that 41.5% were resistant to clindamycin, 38% to moxifloxacin, and 7.9% to rifampin. Resistance to all three antibiotics was present in 27.5% of ribotype 027 isolates but was rare in other ribotypes. (163) Among 316 *C. difficile* isolates from European patients, 48% were resistant to at least 1 of 8 antibiotics tested and 55% of the resistant strains were multi-resistant tolerating 3 or more antibiotics. Mechanisms of resistance were described and the evolution of new resistance patterns was discussed. (158)

### **Infections and carriage in humans**

Incidence of asymptomatic carriage of *C. difficile* in the healthy, general population has been estimated at 3%. However, some populations have a higher rate of carriage. A survey of 100 residents of a home for the elderly (median age 83) found that 10% were asymptomatic carriers of *C. difficile*. (145) A survey of 1234 Japanese adults with no history of antibiotic use during the previous 4 weeks found that 7.6% were asymptomatic carriers of *C. difficile*. (83) After treatment and resolution of symptoms, many CDI cases continue to shed *C. difficile* spores for as long as 4 weeks. (151) These spores are very resistant to sanitizers and environmental stresses and asymptomatic carriers may be sources of hospital- and community-acquired infections.

Early outbreaks of *C. difficile* associated diarrhea (CDAD) or *C. difficile* infection (CDI) occurred in hospitals and epidemiological studies implicated the long-term use of antibiotics in the development of this disease. *C. difficile* is now the most common cause of diarrhea in hospitals and long term care facilities, particularly afflicting those being treated with antibiotics, such as cephalosporins and fluoroquinolones, to control other infections. Antibiotic treatment can destroy much of the normal intestinal flora allowing some resistant bacteria that are normally not very competitive (such as some strains of *C. difficile*) to thrive. Data from a 2010 survey of 89 German hospitals revealed that the incidence of nosocomial *C. difficile* infection was twice that of nosocomial MRSA infection. (113)

Recurrent CDI occurs frequently, generally affecting more than a third of primary cases. An examination of the strains involved in the recurrent infections of 82 persons found that in 51 people, CDI symptoms occurring after an apparent cure were caused by the same *C. difficile* strain, indicating a relapse. In the other patients, a different *C. difficile* strain was detected indicating that a new infection occurred. Infection with 027 was a significant risk for relapse. (102) Several papers from a 2012 symposium discussed different aspects of the problem of recurrent *C. difficile* infections. (47)

In the past 8 years, there has also been an increasing number of cases of *C. difficile* infection occurring outside of hospitals, among younger, healthy persons with no recent history of antibiotic use. (144;50)

### **Disease and Carriage in Animals**

*C. difficile* infects animals as well as humans and differences and similarities in these infections were recently reviewed. (85) Predominant ribotypes of *C. difficile* vary with animal species and geographic location but there is an overlap between animal and human strains. (77;85) *C. difficile* causes diarrhea in piglets (85;154) and horses (15;165). Some isolates from diarrheal pigs and calves are indistinguishable from an important human pathogenic strain, ribotype O78. (41;55;183) Dogs and cats with diarrhea may also harbor *C. difficile*. (32;89) Epidemiology, treatment, and control of enteropathogenic bacteria in dogs and cats were recently reviewed. (101) An outbreak of enteritis caused by *C. difficile* caused the death of two Asian elephants in a zoo in Denmark. It was suggested that the feeding of large quantities of broccoli in the days just prior to illness caused an overgrowth of toxigenic *C. difficile* because broccoli can inhibit the growth of some intestinal microbiota. (20)

Both companion animals and livestock can be asymptomatic carriers of *C. difficile*. See Table 1 for information on surveys of animals that reported the presence of *C. difficile*. In 84 Canadian households with healthy dogs and/or cats, *C. difficile* was detected in 10% of the dogs and 21% of cats. (179) Animals at shelters also harbor *C. difficile*, with 5.5% of dogs and 3.7% of cats at 10 shelters testing positive. (148) This bacterium has been isolated from healthy horses and a year long study of 25 healthy adults revealed that *C. difficile* was shed transiently. (150) Data indicate that *C. difficile* is not usually transmitted directly from animal to animal or vertically from mother to offspring. (69;136)

Younger foals are more often carriers of *C. difficile* than older foals and adult horses. (15) In fact, surveys of healthy farm animals generally show that younger animals, including piglets (116;160;183), hens (185), and calves (34), are more frequently carriers of *C. difficile* than older animals. Newborn piglets delivered by caesarian section test negative for *C. difficile* but within 48 hours all 71 piglets delivered normally and monitored at a Dutch farm became positive for *C. difficile*. *C. difficile* was present on teats of sows, in the air, and on other environmental samples. (69) *C. difficile* carriage generally declines with age as illustrated by a longitudinal study in piglets in Ontario which found a prevalence of 74% at 2 days of age, 56% on day 7, 40% on day 30, 23% on day 44 and 3.7% on day 62. (183)

Presence of *C. difficile* in animals used for food is one indication of the potential for transfer of this pathogen to meat during slaughter and processing of animals. Some recent studies report the presence of this bacterium in 3-5% of feedlot cattle in Canada (33), 6.6% of ruminants (cattle and goats) on 30 Swiss farms (142), 8.6% of Dutch pigs at slaughter (86), 4.9% of turkeys in Italy (146), 2.3% of broiler chickens in Texas (61), and white-tail deer raised on 36.7% of farms tested in Ohio (51). *C. difficile* has also been detected in fecal samples from sheep in the Netherlands. (89) (See Table 1 for more survey results.)

Prevalence of *C. difficile* in livestock may be affected by conditions at different farms. However, several farm specific factors, (conventional vs. organic, more or less than 1000 pigs, finisher farm vs. farrow-to-finish, presence or absence of other livestock) did not appear to significantly affect prevalence. (86) Although one study reported that piglets on an antibiotic free farm had a lower prevalence of *C. difficile* than those on a conventional farm, antibiotic resistant bacteria were present in animals on both types of farms. (160)

## Epidemiology of *C. difficile*

### **Incidence of infection worldwide**

More than 250,000 hospitalizations each year in the U.S. are estimated to be associated with *C. difficile* infection and the economic burden of this illness is close to or may even exceed \$1 billion annually. (43;106) A high incidence of CDI in hospitals significantly increases costs due to longer hospitalization, rehospitalization, more laboratory tests, and more medications. In uncomplicated cases, this may entail an extra \$5000 per patient. But for special populations, for example patients being treated in intensive care units for other illnesses, the increased cost may be as much as \$90,000 in 2008 dollars. (53) The economic burden of CDI is not limited to hospitals. Kaiser Permanente Colorado and NorthWest tracked CDI cases for 3 years and reported that more than half were identified in outpatients, with resulting costs to clinics and to patients who must stay home from work. (93)

During the past 15 years the incidence of CDI in acute care hospitals in the U.S. has increased from 30-40/100,000 to >84/100,000. Data reported by CDC indicate that mortality from CDI has increased steadily from 793 deaths in 1999 to 7,476 deaths in 2008, dropping slightly to 7,284 in 2010. Approximately 91% of these deaths occurred in people aged 65 and older. (115) The increasing severity of illness correlates with the emergence of hypervirulent strains (ribotype O27/NAP1/toxinotype III and ribotype O78 toxinotype V) detected first in the U.S. and Canada in the early 2000s (87;114), then in Europe in 2005, and in Asia, Central America, and Australia in 2008-2010. (31)

Similar recent increases in CDI have also been reported in Canada and Europe. Estimated burden of CDI in Europe is about 5 episodes per 10,000 days of hospital stay. However, this disease is believed to be significantly underreported because clinicians often fail to order tests for *C. difficile* in cases of unexplained diarrhea or else laboratories may use diagnostic tests with low sensitivity. Some CDI cases are missed because symptoms develop after the patient has been discharged from the hospital. Estimates for annual costs for managing CDI in Europe are about €3000 million. (24)

CDI has become a problem in hospitals and communities in other countries and issues related to CDI in Latin America (10) and in Asia (46) were recently reviewed.

Hypervirulent strains produce many more spores and higher levels of toxins than less virulent strains. Infectious dose of *C. difficile* required to cause illness depends on the virulence characteristics of a strain and the susceptibility of the host. There are no data for humans on infectious dose but an experiment with mice demonstrated that exposure to <7 spores/cm<sup>2</sup> of cage space for 1 hour, followed by a dose of clindamycin was sufficient to reproducibly cause illness in healthy animals. While it is not certain how this relates to human infections, it indicates that the infectious dose may be quite low, particularly in those being treated with antibiotics. (97)

### **Healthcare-associated (HA) vs. community-associated (CA) infections**

Traditionally, CDI has been associated with patients who were given broad spectrum antibiotics in hospitals. However, during the past 10-15 years, the epidemiology of *C. difficile* has been changing as the frequency and severity of CDI in humans has increased. Recent reviews discuss the possible roles of the emergence of hypervirulent strains, ageing populations, effects of newer antibiotics, and increased exposure to this pathogen outside of healthcare facilities. (42;50)

Recent trends in the epidemiology of CDI are reminiscent of changes observed in the epidemiology of MRSA (methicillin-resistant *Staphylococcus aureus*) infections. Originally both pathogens primarily affected patients in hospitals and other health care facilities, causing more severe illness in the elderly, the immunocompromised, and those with other significant health problems. Prior treatment with antibiotics was often identified as a risk factor. In recent years, both pathogens have been causing increasing numbers of cases outside of hospitals, among younger, healthy persons with no recent history of antibiotic use. The epidemiology and transmission of these community-acquired infections are not well understood. Although usage of antibiotics and gastric acid suppressants appear to be related to community-associated CDI (CA-CDI), 27% of CA-CDI cases in one U.S. study did not receive antibiotics during the 6 months prior to illness and 17% did not have any risk factors usually associated with CDI. (92)

Increases in CA-CDI have occurred in Europe as well as in the U.S. Reports from CDC in 2005 first described severe infections in 4 states in persons considered at low risk for infection. (30) Surveillance in CT in 2006 found that about 25% of CDI cases had no established risk factors. (130) Records on CDI cases occurring during about 15 years in Olmstead Co., MN were examined to detect differences between CA and HA cases. Incidence of both types of cases increased significantly during this time. CA-CDI cases accounted for about 41% of the total and were younger, more likely to be female and had less severe infections and fewer comorbid conditions. (88)

Recent data from the UK indicates that CDI rates overall have decreased since about 2006-2007, but the proportion of cases acquired in the community has increased. (78) An intensive 3-month study in the Netherlands in 2007-2008 of community onset CDI found that 26% of patients had not been using antibiotics during 6 months previous to infection nor had they been admitted to a healthcare institution within the previous year. Thirteen different PCR ribotypes were detected and some of them had never been detected in hospital outbreaks. (14)

Community-associated infections are defined as those occurring in patients, without hospitalization in the past 3 months and diagnosed in an outpatient clinic or diagnosed within 48 hours of admission to the hospital. If symptoms develop after 48 hours, then the infection was probably acquired in the hospital. Some apparent CA cases at first appear not to have traditional risk factors for infection but on further investigation may have been taking antibiotics or have had close contact with a hospitalized patient. However, for others there are no recognized sources of infection or obvious risk factors. Although *C. difficile* is present in many wild and domestic animals, in water and soil samples and in some foods, there is as yet no direct evidence for the transmission of this pathogen from the environment, foods, or animals to humans. (66) More rigorous identification of *C. difficile* strains from different sources is needed to determine whether humans acquire infections from food or other animals. A recent analysis of isolates from community-associated infections found that they were not related to isolates from food and food animals. (103)

### **Predisposing (Risk) factors for infection**

It has long been recognized that increased age and the use of certain antibiotics are risk factors for CDI. Although nearly all antibiotics have been associated with onset of CDI, exposure to clindamycin, cephalosporins, and fluoroquinolones occurs more frequently prior to onset of symptoms. These broad-spectrum antibiotics alter the normal composition of the intestinal microbiota while certain *C. difficile* strains are unaffected by them. For example, the prominent North American epidemic strain, BI/NAP1, is highly resistant to fluoroquinolones and

clindamycin. (123) A meta-analysis of 5 studies comparing infection with BI/NAP1/027 to infection with other *C. difficile* ribotypes found that age >65 years and prior use of fluoroquinolones were associated with a greater risk for infection with this ribotype. (172)

Antibiotics increase susceptibility to CDI not only when they are being consumed but also for an extended period afterwards. A multi-center case-control study in the Netherlands found that patients were at a 7-10 fold increased risk of CDI for a month after cessation of antibiotic therapy. (65) Experimental studies in mice revealed that a single dose of clindamycin severely reduced the diversity of intestinal microbiota for at least 28 days, allowing the expansion of some microbial species that were previously minor constituents of the microbiota. Inoculation of clindamycin-treated mice with *C. difficile* rapidly provoked diarrhea and colitis and mice remained more susceptible to CDI for at least 10 days after administration of the drug. (25) Antibiotics caused changes in the relative numbers of different microbes in the intestine and the number of different genera of bacteria, thereby reducing diversity of the intestinal microbiota. Fecal *Bifidobacterium* spp. numbers were greatly reduced, particularly by antibiotics that inhibit nucleic acid synthesis (ciprofloxacin, trimethoprim, moxifloxacin). (79;119) Similar effects on intestinal microbiota likely occur in humans treated with antibiotics and may explain the extended period of susceptibility to and alterations in the composition of the intestinal microbiota are associated with CDI. (100;131)

Age-standardized incidence rates of CDI in a population in Iceland were  $\leq 25/100,000$  for those less than 60 years of age. But incidence increased dramatically for those aged 60-79 (128/100,000) and those older than 79 years (319/100,000). (173) However, not all older people and those taking long courses of antibiotics develop CDI. With advent of some new, more virulent strains of *C. difficile*, there has been an increase in the rate of CDI in younger people traditionally thought to be at low risk. Recent studies suggest that other medications and life style factors may also play in susceptibility to CDI.

Use of acid suppressant medications, either alone or in combination with antibiotics, has been proposed as a risk factor that may explain some community- and hospital-associated cases of CDI. (57) *C. difficile* spores can survive normal gastric conditions but vegetative cells are killed by acid. But vegetative cells can tolerate conditions in the stomach if pH >5. (80) A systematic review of 27 studies found that proton pump inhibitors (PPIs) increased gastric pH and increased risk for infections with *Salmonella*, *Campylobacter*, and *C. difficile*. (16) A meta-analysis of 23 studies involving nearly 300,000 patients concluded that there was sufficient evidence that PPI intake increased incidence of CDI. Overall the increased risk was 1.69; for the 17 cohort studies, risk was 2.31 while for the 6 case-control studies, risk was 1.48. (76) Another meta-analysis of 39 studies involving 313,000 patients, found an increased risk of 1.74 associated with use of PPIs and concluded that there was a probable association between PPIs and development of CDI. (94) There was some variability among the various studies included in these analyses with regard to the control of other factors that might be associated with CDI and this could affect the significance of the results.

Data on 16,781 older U. S. individuals (age > 50 years) indicated a correlation between smoking and CDI. While the overall incidence of CDI in this group was 220.6/100,000 person-years, incidence rates for current smokers and never-smokers were 281.6 and 189/100,000 person-years, respectively. Several hypotheses were suggested to explain this effect of smoking. *C. difficile* may be present in cigarettes. Previous studies have documented the presence of *Clostridium* spp. in a high percentage of cigarettes but whether *C. difficile* is a frequent contaminant is unknown. Smokers may contract more infections and therefore use more



antibiotics. Or the gut microbiota of smokers may be different from that of non-smokers and more readily permit growth of *C. difficile*. (141)

### **Potential routes of infection**

Epidemiology and transmission of *C. difficile*, particularly for community-acquired infections, are not completely understood. *C. difficile* is transmitted basically by the fecal-oral route but numerous exposure scenarios are possible. (121) High concentrations of spores ( $10^4$  to  $10^7$  spores/g.) are present in feces of people and animals with active CDI. Prior to treatment about 90% of samples from the skin of hospitalized patients with CDI and of environmental samples in their rooms tested positive for *C. difficile*. Treatment caused resolution of diarrhea in an average of 4.2 days. Yet some patients and their environments still contained spores 6 weeks after treatment. (151)

### **Person to Person Contact**

*C. difficile* is commonly present on the skin of patients with CDI, with highest counts generally present on the abdomen and lowest counts on the chest. Spores were readily transferred to moist gloved hands touching the skin of patients. It is believed that the hands of healthcare workers are an important means of transporting nosocomial pathogens throughout hospitals and other health facilities. (56) However, a study of the transmission of *C. difficile* in hospital wards at a large U.S. hospital indicated that transmission from patients with CDI was not sufficient to sustain transmission to other residents of the ward, Rather, admission of new colonized patients was an important factor in sustaining transmission. (95)

Asymptomatic carriers may be an important source of *C. difficile* in the community and in long term care facilities. More than half of 68 asymptomatic residents at one facility were found to be carriers and *C. difficile* was present on their skin and in their environment. Spores on the skin were easily transferred to the hands of others suggesting that personnel attending these residents can spread *C. difficile* to other residents and areas of the facility. (132)

Persons working in environments where they are routinely exposed to *C. difficile*, such as nurses, day care workers, some farm workers, and some persons working in veterinary clinics, may transport spores on their clothing from the workplace into their homes and the community. (121) Infants at day nurseries are often colonized with *C. difficile* and sometime secrete spores for several months. (143) Some data from Canada indicated that direct transmission of *C. difficile* from CDI cases to family members was not very common. (126)

### **Animal to Person Contact**

Healthy animals of several species carry and intermittently shed *C. difficile* suggesting the possibility that humans may be infected by direct contact with companion animals or livestock in occupational settings or at fairs and petting zoos. A Canadian survey detected *C. difficile* in 10% of dogs. However, ribotype analysis of canine and home environmental strains indicated that dogs were not a significant source of household contamination. In fact, some dogs may acquire *C. difficile* from humans as living with an immunocompromised individual was associated with colonization in dogs. (179) Therapy dogs that visit human healthcare facilities may acquire *C. difficile* from contact with patients or contaminated floors. (98) If this is a frequent occurrence, the these dogs may be a mechanism for transporting *C. difficile* within these facilities and possibly exposing other patients/residents to this pathogen.

An examination of fecal samples from goats, sheep, calves, pigs, ponies, a rabbit, and a donkey at 4 Dutch petting zoos revealed the presence of *C. difficile* in only one pig. This suggests that there is not a great risk of infection to visitors at the zoos although *C. difficile* spores could contaminate and remain in these environments for months. (71) Another study of 158 4-H members and 203 of their animals found *C. difficile* in 13 people but not in any of their animals. *C. difficile* was isolated from 2 horses and 1 pig but not from their human caretakers. This is further evidence that fair visitors are not at increased risk for acquisition of *C. difficile*. (107)

A study at a closed swine operating system in Texas investigated *C. difficile* isolates from pigs, from workers who had direct contact with pigs and from workers who were not directly exposed to pigs. No difference in prevalence of *C. difficile* carriage was observed in the two groups of workers, suggesting that direct exposure to pigs was not an important route of transmission. However, *C. difficile* isolates from pigs and workers were very similar indicating that there may have been airborne transmission of spores leading to general environmental contamination in the facility. If spores were not transmitted from one species to another, then both species may have been exposed to some other common source of *C. difficile*. (117)

Animals may be vectors for transporting *C. difficile* around farms. House mice, house sparrows and some insects (drain flies, lesser house flies and mealworms) on a pig farm in the Netherlands tested positive for *C. difficile* as did some samples of bird droppings. Piglets acquire *C. difficile* infections from the environment and these pest animals may increase the range of environmental sources for infection. (26)

### **Airborne Transmission**

In spite of intensive cleaning and sanitation efforts, many surfaces in health care facilities test positive for *C. difficile*. A short pilot study examining the possible role of aerial transmission of this bacterium detected *C. difficile* spores at concentrations of 53-426 cfu/m<sup>3</sup> air during 2 days of sampling. No spores were detected during another 2 days indicating that aerial transmission may be sporadic. (133)

Air sampling (for 1 hour) near 50 patients with CDI revealed the presence of *C. difficile* around 6 patients. However, when sampling time was extended to 10 hours over 2 days, air around 7 of 10 patients tested positive. Contamination was more frequently detected during times of increased activity, for example during the busy lunch hour. Molecular characterization of isolates confirmed a link between airborne dispersal and environmental contamination. (17)

When a fecal suspension containing 10<sup>7</sup> spores/ml was flushed in lidless toilets of the type often used in health care facilities, *C. difficile* spores were detected in air samples up to 25 cm above the toilet seat. Further, a range of 15-47 droplets were emitted during flushing. These airborne droplets and spores could settle and remain on surfaces in the surrounding area. (18)

Spores are also present in the air around farrowing pens at farms with animals carrying *C. difficile*. Peak spore counts were detected around the time that farm workers were active in the area. Airborne spore dispersal was detected as far as 20 m from the farm. (84)

### **Contact with Contaminated Equipment and Surfaces**

Infected patients discharge large numbers of *C. difficile* spores and vegetative cells during diarrheal episodes and these spores and cells may be deposited on numerous surfaces in the environment. Vegetative cells can survive up to 6 hours on moist surfaces while spores are

very resistant to environmental stresses and to some classes of sanitizers and may therefore persist for extended periods (up to several months) in the environment. (39;80)

Spores of toxigenic *C. difficile* have been detected on surfaces in patients' rooms, portable pieces of equipment, and doctors' and nurses' work areas. (44;90;122) Stethoscopes can pick up *C. difficile* from the skin and transfer the spores to another surface. (170) Hospital curtains can also be contaminated with pathogens and hand imprint cultures demonstrated that they could readily transfer to hands touching the curtains. (168)

### **Consumption of Contaminated Food or Water**

Since *C. difficile* is spread by the fecal-oral route and has been isolated from livestock and poultry, there is a potential for foodborne transmission as a route for human infection. Meat could be contaminated during slaughter of animals carrying *C. difficile* and could also be contaminated by food handlers who are carriers and do not practice good personal hygiene. Surveys in North America and Europe have found low levels of *C. difficile* in a small percentage of retail meat, including beef, veal, chicken, turkey, pork, lamb, and some sausage. Generally, the reported prevalence of *C. difficile* is higher in meat from North America than in meat from Europe. *C. difficile* was also detected in dog food containing raw turkey. (181) Some salad vegetables, root vegetables, and seafood were also found to be contaminated. One report from 1988 indicated that spores of *C. difficile* and other clostridia were present in honey. (128) Table 2 summarizes results from published studies that detected *C. difficile* in human foods. Although only low levels of spores were detected, some strains of *C. difficile* in foods are toxigenic and are similar to strains isolated from cases of human illness. Other published research reported the absence of *C. difficile* in meats (23;40;68;75;82;99;175), seafood (110), and raw milk (82).

Significance of the presence of *C. difficile* spores in food is unclear at present. There is no data on infectious dose and it is likely that this depends on the health of individuals and whether they have recently been exposed to broad spectrum antibiotics. Although early descriptions of *C. difficile* indicated that it did not produce lipases and could not digest meat and milk, recent preliminary data described growth on media containing meat or fish juice and on ground beef. The authors did not report whether toxins were produced during growth. (159) An in vitro study indicated that the structures of toxins A and B are partially unfolded at 40-45°C although they tended to be more stable at higher pH values. (147) This suggests that the activity of toxins (if they were present in foods) would be destroyed by cooking. However, the stability of the toxins in the presence of various food constituents has not been determined. If spores are present in meat or other foods, they would not be destroyed by ordinary cooking to recommended temperatures. (138)

## **Control and Prevention of CDI**

### **Hospital and healthcare programs**

Following some severe outbreaks of CDI in healthcare facilities, comprehensive control programs have been instituted at some hospitals to reduce the spread of *C. difficile* among vulnerable patients. According to data collected by CDC during 2010, 52% of 42,157 CDI cases treated at hospitals had symptoms at admission. Patients with diarrhea may excrete over a million spores per gram of feces and these may contaminate the environment and infect other patients. Therefore, early and reliable detection of CDI and isolation of symptomatic patients are

important steps in limiting the spread of infection. Attention to handwashing, the use of gloves and proper cleaning and sanitation of rooms, instruments and frequently contacted surfaces can eliminate or reduce the potential for transmission of *C. difficile*. Hospitals in three states that aggressively implemented these programs saw their CDI rates decline by at least 20%. (105)

A multipronged infection control program at a Canadian hospital that included monitoring and reducing the use of broad-spectrum antibiotics where possible and the hiring of infection control practitioners along with increased cleaning and housekeeping and the rapid identification and isolation of CDI cases achieved a 61% reduction in CDI cases. (184) A significant decrease in CDI was observed in another hospital after implementation of a program that reduced use of broad spectrum antibiotics in favor of “low risk” antibiotics. (161) Other recent reviews discussed important aspects of antimicrobial stewardship and hospital infection control programs. (74;123;176) Cost effectiveness of screening and isolation programs were estimated in a simulation model. (13)

Identification of patients with diarrhea caused by *C. difficile* rather than by some other pathogen can be time consuming because of slow growth on culture plates. Some researchers in the Netherlands trained a dog to detect the odor of *C. difficile* and the dog was able to correctly identify 25 of 30 patients with CDI and 265 of 270 control patients without CDI by walking past their hospital beds. (21)

Although public reporting of the quality of care in hospitals is controversial, a study in Canada found that mandatory public reporting of hospital-acquired infection rates was associated with a 26.7% decrease in CDI. The direct actions within the hospital that effected this change were not described but there was apparently an incentive to improve infection control practices. (37) Improved education for housekeeping staff and monitoring the effectiveness of cleaning and disinfection practices to provide feedback to frontline staff has been found to result in significantly fewer isolates of *C. difficile* from surfaces in health care facilities. (45)

### **Sanitizers and surface treatments**

Bacterial spores, including those of *C. difficile*, can survive on dry, inanimate environmental surfaces for at least 5 months. (90) But vegetative *C. difficile* cells die quickly on dry surfaces. However, cells may remain viable for up to 6 hours on moist surfaces in rooms such as bathrooms and kitchens. (80) Prior to treatment for CDI, patients excrete 10 times as many vegetative cells as spores but once antibiotic treatment starts, spores are the primary form detected. Numerous surface cleaning and sanitation methods have been devised to kill infectious *C. difficile* on surfaces, as described in these articles. (36;49)

Bleach solutions are recommended as effective sanitizers rather than alcohol wipes or quaternary ammonium compounds because spores are inactivated more readily by chlorine. Some recent studies provided evidence for the efficacy of bleach in reducing CDI. Following a new program for thorough cleaning, using dilute bleach, of all surfaces in hospital rooms occupied by CDI patients after patients were discharged, resulted in a 48% reduction the average number of CDI patients per 1000 patient-days in the hospital as compared to the preintervention rates of illness. (58) Germicidal bleach wipes used for daily cleaning on wards with a high rate of hospital acquired CDI reduced CDI incidence in the hospital by 85%. (120) However, a chlorine dioxide based cleaning system was not effective in reducing contamination of surfaces or CDI rates in a hospital trial. (54)

Despite its effectiveness, bleach can be irritating and unpleasant to use and corrosive on some surfaces and equipment. One alternative, peracetic acid sporicidal wipes, did remove

significant numbers of *C. difficile* spores from surfaces and reduced spore counts on hands of health care workers and rates of CDI. (28;91) A series of 32 disinfectants, tested against *C. difficile* spores, varied in their effectiveness with some requiring an hour of contact time before significant reductions were observed. Generally, compounds containing chlorine performed the best. The presence of other organic matter (dirt) also affected the efficacy of the disinfectants. (157)

Other proposed disinfectants are gaseous compounds, including steam, hydrogen peroxide, chlorine dioxide, and ozone. (38) Use of a portable saturated steam vapor disinfection system reduced counts of *C. difficile* spores dried on a surface to undetectable levels in 5 seconds. However, low concentrations of spores were used, so this system requires further testing. (162) An accelerated hydrogen peroxide cleaner killed spores in and on toilets used by CDI patients, reducing levels to 28% of untreated toilets. (3) Hydrogen peroxide vapor has also been used to clean hospital rooms achieving a 6 log reduction in 2-3 hours. (52;63) Another room disinfection system, utilizing 80 ppm ozone and 1% hydrogen peroxide achieved 6 log reductions in spores in a shorter time of 60-90 minutes. (186)

Nonthermal atmospheric gas discharge plasmas can sterilize surfaces without leaving a chemical residue behind. In tests with spores of several *Clostridium* and *Bacillus* species, the plasma inactivated *C. difficile* spores with a D value of 2.8 min. Other clostridia were more resistant to the plasma. (169)

### **Prevention of Animal Disease**

*C. difficile* infection can be a significant problem in neonatal swine. Following some encouraging results in laboratory animals, an attempt was made to protect piglets from CDI by administering a nontoxigenic strain of *C. difficile* to them either by direct inoculation of each piglet or by spraying the perineum and teats of dams with spores of this strain. More piglets were weaned from treated litters and their average weaning weight was higher than that of piglets from untreated litters. Analyses of fecal samples from piglets 5 days after birth showed the presence of *C. difficile* toxins in 58.3% of control litters, 44.8% of sprayed litters and 13.8% of litters in which piglets received a direct dose of non-toxigenic *C. difficile*. Further research may determine whether this is a practical strategy for protecting piglets. (155)

### **Prevention of foodborne intoxication or infection**

Although some preliminary data indicates that *C. difficile* can grow on media containing meat or fish juice and on ground beef (159), this bacterium, like other clostridia, is an anaerobe and does not grow in the presence of oxygen. It is not clear whether there are foods that will support growth and toxin production by *C. difficile* or whether the critical issue is simply the number of spores deposited on foods at the point of contamination. It appears that the structures of toxins A and B are partially unfolded at 40-45°C (although they tended to be more stable at higher pH values). (147) This suggests that the activity of toxins (if they were present in foods) would be destroyed by cooking. However, the stability of the toxins in the presence of various food constituents has not been determined. If spores are present in meat or other foods, they would not be destroyed by ordinary cooking to recommended temperatures. (138)

Particularly for foods to be served to vulnerable populations in hospitals, nursing homes, and elsewhere, efforts should be made to prevent contamination throughout the food production, processing and preparation chain. Procedures used to reduce contamination with other intestinal pathogens, for example *E. coli* and *Salmonella*, during slaughter and processing of livestock will

aid in diminishing *C. difficile* on carcasses and pieces of meat although spores will survive heat and some cleaning/disinfection steps that would inactivate vegetative pathogens. Workers in food processing and preparation have been implicated in outbreaks of foodborne disease. They may shed bacteria and viruses, even when asymptomatic and several weeks after they have recovered from an illness. Improved hygiene precautions, consistently practiced by persons in food preparation and processing would significantly improve safety of foods. (167)

### **Data gaps and research needed**

Further information and research is needed to determine whether *C. difficile* in food presents a significant threat to human health.

- The few studies that have reported concentrations of spores in foods indicate that contamination levels are very low. More data is needed on spore levels in different foods.
- There is not yet any good data on the possible growth and toxin production of *C. difficile* in different foods. Although the presence of *C. difficile* in many foods may not be a risk for illness, there may be certain foods that are more commonly or heavily contaminated with *C. difficile* or environmental conditions that support growth of *C. difficile* and toxin production as there are for *C. botulinum* and *C. perfringens*.
- *C. difficile* spores are known to survive ordinary cooking temperatures but further information is needed on the ability of these spores to survive other processing conditions and antimicrobials that may be added to foods.
- Nor is there enough information on the stability of toxins A and B at different temperatures or pH values in foods.
- More rigorous genotyping methods are needed to determine whether *C. difficile* strains present in animals, food, or environmental samples are the same as those isolated from human CDI cases.
- The infectious dose of *C. difficile* in healthy persons or in those whose normal microbiota has been depleted by antimicrobial use or those with other comorbid conditions is unknown.

Although we know that chlorine-based disinfectants can kill *C. difficile* spores, hospitals and other healthcare facilities continue to experience problems with CDI. More research may be needed on other effective disinfectants. Hospitals and other institutions with continuing contamination problems may need to devise better organized systems for cleaning and disinfection. This may involve: more rapid identification of contaminated areas, prevention of contamination of instruments, minimizing production of aerosols containing *C. difficile* spores, and educating and encouraging personnel to adhere to strict infection control procedures. These strategies will also be important for food processors if *C. difficile* is determined to be a foodborne pathogen.

### **Summary and Perspective**

During the past 10-15 years, the frequency and severity of CDI in humans has increased. While it is still true that the majority of infections occur in hospitals and other healthcare facilities, in people over 65 years old, in those taking certain antibiotics such as fluoroquinolones and in persons with other serious health issues, an increasing number of younger, healthy and non-hospitalized persons have recently developed CDI. The emergence of hypervirulent strains,

ageing populations, newer wide-spectrum antibiotics, and increased exposure to *C. difficile* outside of healthcare facilities may all have played a role in this changing epidemiology.

The normal habitat of *C. difficile* is the gastrointestinal tract of humans and other animals (including livestock and companion animals). As such, large numbers of spores are present in feces of infected people and animals as well as of asymptomatic carriers. Therefore, infection of new hosts occurs by some version of the fecal-oral route. Although *C. difficile* has been detected in many domestic animals, in water and soil samples and in some foods, there is as yet no direct evidence for the transmission of this pathogen from the environment, foods, or animals to humans.

There are many unanswered questions about the epidemiology of this pathogen and it would be wise to monitor ongoing research on this organism to determine whether it poses a risk as a foodborne pathogen.

Table 1. Surveys reporting *C. difficile* in animals (Unless indicated, animals were healthy.)

Animal	Number tested	% positive	Cd strains	Location	Year reported	Reference
Calves	18	22.2	078	Belgium	2012	(134)
Calves	47	12.7	003, 033, 066	Switzerland	2012	(142)
Calves, veal	100	6	012, 033	Netherlands	2012	(89)
Calves, veal	200	28		Pennsylvania	2012	(73)
Calves, veal	200	61	078, 11 others	Canada	2011	(34)
Calves, veal	71	33.8		Pennsylvania	2010	(72)
Calves, veal	204	0.49	078	Switzerland	2010	(68)
Calves, veal	42	9.5	033	Slovenia	2009	(6)
Calves, veal, diarrheic	253	25.3	078, 017, 027,	Southwestern	2008	(60)
healthy	53	12.7	5 others	U.S.		
Calves, veal	56	1.8	066	Slovenia	2008	(127)
Calves, veal, diarrheic	144	7.6	017, 027, 5	Canada	2006	(140)
healthy	134	14.9	others			
Cattle, at harvest	202	6.9	078	Belgium	2012	(134)
Cattle	874	4.1	078	Canada	2012	(33)
Cattle, dairy	63	1.5	137	Switzerland	2012	(142)
Cattle, dairy	100	1	012	Netherlands	2012	(89)
Cattle, at harvest	944	1.8	078, 3 others	U.S.	2011	(135)
Cattle, arrival at feedlot	186	12.9	078, 1 other	Canada	2011	(136)
Prior to harvest	186	1.2				
Cattle, Beef	2965	6.3		U.S.	2011	(166)
Dairy	1325	2.4				
Cattle, at harvest	67	4.5		Austria	2009	(75)
Cats	135	3.7	010, 014/020, 039, 045, SLO 066	Germany	2012	(148)
Cats, diarrheic	115	15.7	014	Netherlands	2012	(89)
Cats	14	21	001	Canada	2010	(179)
Cats, hospitalized	42	7.1	11 ribotypes	Canada	2008	(32)
Cats	100	2		U.K.	1996	(1)
Cats	20	30			1983	(22)
Chickens	300	2.3	NAP7	Texas	2011	(61)
Chickens	61	62.3	12 PCR ribotypes	Slovenia	2008	(185)
Chickens	100	29		Zimbabwe	2008	(152)
Chickens, at harvest	59	5		Austria	2009	(75)
Poultry, Healthy	100	5.0	014, 002, 045	Netherlands	2012	(89)
Diarrheic	21	9.5				
Poultry, mixed	120	1.2		U.K.	1996	(1)
Deer	30*	36.7	078	Ohio	2010	(51)
Dogs	165	5.5	010, 014/020, 039, 045, SLO 066	Germany	2012	(148)
Dogs, diarrheic	116	25	014, 012, 021, 107	Netherlands	2012	(89)
Dogs	139	10	001	Canada	2010	(179)
Dogs, hospitalized	360	19	11 ribotypes	Canada	2008	(32)



Dogs	100	10		U.K.	1996	(1)
Dogs	52	21		U.K.	1983	(22)
Duck	2	50		U.K.	1983	(22)
Goats	40	7.5	001, 066	Switzerland	2012	(142)
Goose	2	50		U.K.	1983	(22)
Horses	15	53.3		Canada	2012	(149)
Horses, Diarrheic	135	17.8	014, 012, 005, 078, 5 others	Netherlands	2012	(89)
Horses	20	5	033	Slovenia	2009	(6)
Horses, diarrheic	62	23	012	Australia	2011	(165)
Horses	38	44	12 PCR ribotypes	Canada	2007	(5)
Horses, Mature, healthy	320	0.3		Sweden	2003	(15)
Mature, enteric disorder	180	12.2				
Foals, <14 days	56	29				
Foals, >14 days	170	1.76				
Horses	100	1		U.K.	1996	(1)
Piglets	23	78.3	078	Belgium	2012	(134)
Piglets, Conventional	350	34	Toxinotype V	North Carolina	2012	(160)
Antimicrobial-free	244	23				
Piglets, scouring				U.S. Midwest	2010	(8)
Large, integrated system	333	57.7				
Smaller regional farms	180	27.2				
Piglets, Healthy	287	28.6		Spain	2009	(4)
Diarrheic	254	22.8				
Piglets	122	50		Texas	2009	(116)
Piglets	485	50.9	066, 029, SI 011, SI 010	Slovenia	2009	(6)
Piglets	257	51.8		Slovenia	2008	(127)
Pigs, Healthy	100	0	078, 023, 005	Netherlands	2012	(89)
Diarrheic	36	25				
Pigs, adult	345	15.9		U.S.	2011	(166)
Pigs, at harvest	436	6.9	078	Canada	2011	(182)
Pigs, at harvest	677	8.6	078, 15 others	Netherlands	2011	(86)
Pigs, at harvest	50	28	015, 6 others	Netherlands	2011	(70)
Pigs, at harvest	61	3.4		Austria	2009	(75)
Pigs, lactating sows	143	23.8		Texas	2009	(116)
Pigs, feral	161	4.4		U.S.	2011	(164)
Sheep, diarrheic	11	18.2	015, 097	Netherlands	2012	(89)
Sheep	100	1		U.K.	1996	(1)
Turkey	82	4.9		Italy	2009	(146)

- Pooled samples from 30 farms

Table 2. Reports of *C. difficile* detected in foods

Food	Samples tested	% positive	Spore concentration	Cd strains	Location	Year sampled	Ref
Beef, ground	115	12	≤10 – 240 spores/g	027, 078	Canada	2008	(178)
Beef, ground	105	1.9	>2 CFU/5g	012	France	2008	(23)
Beef, ground	32	6.25			Sweden	2008	(175)
Beef, ground, retail	24	8.3		0088, 0348	Manitoba	2007	(174)
Beef, ground	26	50		027, 078	Arizona	2007	(156)
Beef, ground	149	6.7		027, 077, 014	Canada	2006	(137)
Beef, ground	53	20.8		M31, 077, 014, M26	Canada	2005	(139)
Pork and beef, ground	70	4.3		AI-57, 053	Austria	2007-08	(82)
Pork, ground	34	38	<0.18 – 0.45 spores/g	078	Pennsylvania	2011	(35)
Pork	243	9.5		078	Texas	2008-09	(62)
Pork, ground	115	12	≤10 – 60 spores/g	027, 078	Canada	2008	(178)
Pork, ground, retail	24	4.2		0139	Manitoba	2007	(174)
Pork, chops and ground	393	1.8		027	Canada	2007-08	(111)
Pork, ground	7	42.9		027, 078	Arizona	2007	(156)
Pork, sausage	13	23.1		027, 078	Arizona	2007	(156)
Veal, ground	50	8	Toxin detected		Pennsylvania	?	(73)
Veal, chops	65	4.6		027	Canada	2006	(137)
Veal, ground	7	14.3		M31, 077, 014, M26	Canada	2005	(139)
Lamb	16	6.3		045	Netherlands	2008-09	(40)
Poultry	32	12.5		078	Texas	2010	(62)
Chicken	203	12.8	10 – 99 spores/g	078	Canada	2008-09	(180)
Chicken	257	2.7		001, 003, 087, 071	Netherlands	2008-09	(40)
Turkey, ground	9	44.4		078	Arizona	2007	(156)
Sausage, Summer	7	14.3		027	Arizona	2007	(156)
Sausage, Braunschweiger	16	62.5		027, 078	Arizona	2007	(156)
Sausage, Chorizo	10	30		027, 078	Arizona	2007	(156)
Fish, Perch	2	50		078	Canada	2010	(110)
Fish, Salmon	20	5		078	Canada	2010	(110)
Shellfish, Clams and mussels	52	49		Multiple types, not 078 or 027	Italy	2010-11	(124)

<b>Food</b>	<b>Samples tested</b>	<b>% positive</b>	<b>Spore concentration</b>	<b>Cd strains</b>	<b>Location</b>	<b>Year sampled</b>	<b>Ref</b>
Shellfish, Clams and mussels	6	67		005, 010, 066	Italy	2009	(125)
Shellfish, Scallops	3	33		078	Canada	2010	(110)
Shellfish, Shrimp	3	33		078	Canada	2010	(110)
Salads, packaged	40	7.5	<3.0 CFU/g	017, 001	Scotland	2008	(9)
Vegetables	111	5		078	Canada	2009	(112)
Vegetables	300	2.3			Wales, UK	1995	(1)

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