Chapter 3

Clostridium difficile infection in the community:

a zoonotic disease?

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Clin Microbiol Infect 2012

Abstract

Clostridium difficile infections (CDI) are traditionally seen in elderly and hospitalized patients who have used antibiotic therapy. In the community, CDIs requiring a visit to a general practitioner are increasingly occurring among young and relatively healthy individuals without known predisposing factors. *C. difficile* is also found as a commensal or pathogen in the intestinal tracts of most mammals, and various birds and reptiles. In the environment, including soil and water, *C. difficile* may be ubiquitous; however, this is based on limited evidence. Food products such as (processed) meat, fish and vegetables can also contain *C. difficile*, but studies conducted in Europe report lower prevalence rates than North America. Absolute counts of toxigenic *C. difficile* in the environment and food are low, however the exact infectious dose is unknown. To date, direct transmission of *C. difficile* from animals, food or the environment to humans has not been proven, although similar PCR ribotypes are found. We therefore believe that the overall epidemiology of human CDI is not driven by amplification in animals or other sources. As no outbreaks of CDI have been reported among humans in the community, host factors that increase vulnerability for CDI might be of more importance than increased exposure to *C. difficile*. Conversely, emerging *C. difficile* type 078 is found in high numbers in piglets, calves and their immediate environment. Although no direct evidence proving transmission to humans, circumstantial evidence points towards a zoonotic potential of this type. In future emerging PCR ribotypes, zoonotic potential needs to be considered.

Introduction

Clostridium difficile is an anaerobic, spore-forming bacterium that can produce toxin A or B upon colonization of the gut. Patients at risk for *C. difficile* infection (CDI) subsequently develop diarrhoea or, in severe cases, a pseudomembranous colitis. Traditionally, elderly and hospitalized patients who had used antibiotic therapy were considered to be the most vulnerable to CDI[3]. Because these high risk patients are primarily located in healthcare facilities, CDI was regarded as a primarily nosocomial disease for many years. This concept is now being challenged, because persons outside hospitals are increasingly developing CDI[4-7].

When CDI is acquired in a healthcare facility, symptoms may start during hospitalization, but they may also develop after discharge. Subsequently, 25 to 50% of the patients who develop CDI outside a hospital have had a recent hospital admission[5, 8-10]. A clear definition of CDI is necessary to distinguish between healthcare-acquired CDI and community-acquired CDI (CA-CDI). For this review, we define CA-CDI as follows: patients with symptoms of CDI starting in the community or within 48 hours of admission to a healthcare facility, provided that the onset was more than 12 weeks after the last discharge from a healthcare facility, according to guidelines from the European Centre for Disease Prevention and Control and the CDC[1, 2]. Some studies included in this review have modified this definition (Tabel 1).

Besides its presence in humans, *C. difficile* has also been described as a commensal or pathogen in numerous animal species. Because patients with CA-CDI do not, by definition, acquire *C. difficile* in a hospital, the question arises as to what the source of exposure might be in the community. Direct or indirect contact with animals was proposed as a possible source of *C. difficile*. This review describes the occurrence of CA-CDI and discusses the potential sources of *C. difficile* in the community. Furthermore, it summarizes the evidence for *C. difficile* being considered as a new zoonotic agent.

Table 1. Incidence of Clostridium difficile infections in the community and the association with antibiotic usage.

			CDI patients						
Author	**Study period**	**Setting**	**(N)**	**Incidence**	**Population tested**	**Definition of CA-CDI**	**Test for C difficile**	**Prior antibiotic usage**	**Median age CDI patients (y)**
Riley et al.[25]	1983-1984	GP/small hospital, Australia	89 (36 toxi-genic)	4.7% (2.1% toxigenic)	Diarrhoeal samples submitted by GP/hospital	CO-CDI	Culture, followed by cytotoxicity assay	-	-
Riley et al.[26]	1988	GP, Australia	16	5.6%	Diarrhoeal samples submitted by GP	CO-CDI	Selective enrichment broth and latex agglutination test	69% (3 months)	-
Hirschhorn et al.[17]	1988-1990	GP/ hospital, USA	51	7.7 / 100,000	Upon request	CO-CDI or onset within 48 hours of admission, no hospitalisation previous 6 weeks, or diagnosis and symptoms within 5 days of admission	Cytotoxicity assay	65% (42 days)	37
Khanna et al.[7]	1991-2005	GP/ hospital, USA	157	2.8 / 100,000 in '91-'93 15 / 100,000 in '03-'05	Upon request	CO-CDI, or onset within 48 hours of admission, no hospitalisation previous 4 weeks	Cytotoxicity assay, switched to enzyme immunoassay	78% (3 months)	50 (mean)
Barrett et al.[18]	1994	GP, UK	7	5.8%, 13.6% (2 districts)	Upon request	CO-CDI	-	-	-
Riley et al.[24]	< 1994	GP, Australia	13 62	2.6% 10.7%	Feacal samples submitted by GP Upon request or after antibiotic use	CO-CDI, no recent hospitalisation CO-CDI, no recent hospitalisation	Vero cell assay and culture	85% (4 weeks)	43 (mean)
Wheeler et al.[22]	1993-1997 1993-1997	commu-nity, UK GP, UK	6 17	160 / 100,000 20 / 100,000	Diarrhoeal patients Diarrhoeal patients	CO-CDI CO-CDI	Vero cell assay Vero cell assay	- -	- -
Karlstrom et al.[13]	1995	GP/ hospital, Sweden	529	20 / 100,000	Upon request	CO-CDI, no hospitalisation previous 4 weeks	Enzyme immunoassay, sometimes with culture	88% (6 weeks)	59
Dial et al.[5]	1994-2004	GP, UK	1233	<1 / 100.000 in 1994 18 / 100.000 in 2004	Upon request	CO-CDI no hospitalisation previous year	Non-specified toxin test	36% (3 months)	71 (mean)

Author	Study period	Setting	(N)	CDI patients Incidence	Population tested	Definition of CA-CDI	Test for C difficile	Prior antibiotic usage	Median age CDI patients (y)
Wilcox et al.[23]	1999	GP, UK	42	24 / 100,000	Diarrhoeal samples submitted by GP	CO-CDI	Vero cell assay and culture	52% (1 month)	<65
Noren et al.[12]	1999-2000	GP/ hospital, Sweden	59	25 / 100,000	Upon request	CO-CDI, no hospitalisation during study period	McCoy cell assay and culture	-	64
Forward et al.[21]	1999-2000	GP, UK	5	0.6%	Diarrhoeal samples submitted by GP	CO-CDI	Vero cell assay	100% (1 month)	>60
Hirshon et al.[27]	2002-2007	primary care clinic/ hospital, USA	43	3.9%	Diarrhoeal outpatients	CO-CDI	Enzyme immunoassay	63% (1 month)	44 (mean)
Kuntz et al.[19]	2004-2007	GP/ hospital, USA	304	11 / 100,000	Upon request	CO-CDI, no hospitalisation in preceding 12 weeks	-	73% (6 months)	between 19-49
Lambert et al.[14]	2005-2006	GP/ hospital, Canada	275	23 / 100,000	Upon request	CO-CDI, or onset within 48 hours of admission, no hospitalisation previous 12 weeks	Enzyme immunoassay	-	<60
Anonymous (MMWR)[20]	2006	GP/ hospital, USA	241	6.9 / 100,000	Upon request	CO-CDI, or onset within 48 hours of admission, no hospitalisation previous 12 weeks	Non-specified toxin test	68% (3 months)	between 45-64
Huhulescu et al.[29]	2007	GP, Austria	14	236 / 100,000 4.6%	Diarrhoeal patients	CO-CDI	Culture followed by immunocard	38% (2 months)	36
Bauer et al.[32]	2008	GP, the Nether-lands	37	1.5%	Diarrhoeal samples submitted by GP	CO-CDI	Enzyme immunoassay	58% (6 months)	54
Fellmeth et al.[15]	2008-2009	GP, UK	54	1.5% 13 / 100,000	Diarrhoeal samples submitted by GP of patients <65 years	CO-CDI	Enzyme immunoassay	32% (4 weeks)	between 31-40

CA-CDI, community-acquired CDI; CO-CDI, CDI that started in the community. Studies that used this definition only, disregarded the presence of a recent hospital admission. GP: general practice.

Occurrence of CDI in the community

CDI is frequently diagnosed within healthcare facilities, and the incidence can rise above 200 per 10,000 admissions[11]. The incidence of CDI occurring outside healthcare facilities is significantly lower[12]. Nevertheless, CDI acquired in the community accounts for one-quarter of all diagnosed CDI patients[7, 12, 13]. Table 1 summarizes studies investigating the incidence of CA-CDI, and shows the study population, the definition of CA-CDI applied, the test that was used, and the incentive to test patients for CDI. In four studies, an enzyme immunoassay was used to diagnose CDI. Enzyme immunoassays have been criticized recently for their low sensitivity, despite their good specificity[10, 13-15]. However, even a relatively specific test will give false positive results in a low-incidence setting such as the community[16], and this will impact on a reliable estimation of the incidence. The incentive to test for CDI also affects the incidence. When patients are tested only upon request of the physician, patients without known risk factors for CDI may be missed. Half of the studies reported in Table 1[5, 7, 12-14, 17-21] only tested faeces samples for *C. difficile* on request of the physician. Despite the relatively similar incidence rates that are reported, most studies are likely to under-report the real occurrence of CDI, owing to their methodological weaknesses.

The population-based study by Wheeler et al.[22] was the only study that tested all diarrhoeal patients in the community, regardless of whether patients visited a general practitioner (GP) or whether CDI was suspected. Between 1993 and 1997, they included 9776 patients, randomly selected from the GPs' patient lists, and prospectively questioned them about the occurrence of diarrhoea during six consecutive months. Diarrhoea occurred in 781 cases and six of these patients were found to be positive for *C. difficile* by the use of Vero cells, resulting in an incidence of 160 per 100,000 persons per year. Microbiological studies in the community are scarce, and all other studies in Table 1 were performed among patients with diarrhoea visiting their GP. These studies report an incidence of CDI of 7 to 25 per 100,000 persons per year[5, 12-14, 17, 19, 20, 22, 22, 23], which is eight-fold lower than the incidence found in the community. This difference suggests that many patients do not seek medical attention for mild diarrhoea caused by *C. difficile*[22]. When the number of people serviced by a laboratory or hospital (catchment area) is unknown, incidences cannot be determined, and only the percentage of positive tests can be reported. Patients presenting to the GP with diarrhoea have a positive test result for CDI in 2 to 6% of the cases[10, 18, 24-27]. This increases to approximately 10% when

antibiotics are used or a physician specifically requests testing, often because risk factors are present[24, 28].

Campylobacter, *Salmonella*, *Yersinia* and *Shigella* were more frequent causes of diarrhoea diagnosed by GPs, according to Wheeler et al.[22] An Australian study detected *C. difficile* in 89 patients, and 36 strains produced toxins (2.1% of total). Toxigenic *C. difficile* was also less frequent than *Campylobacter* (3.2%), *Shigella* (3.2%) and *Salmonella* (2.9%) in this study.[25] Both studies were performed before the incidence of CDI increased worldwide in the beginning of the 20th century. In 2007, a small Austrian study concluded that CDI was the most frequent bacterial cause of gastroenteritis in general practice.[29] However, this finding should be interpreted with caution as the incidence of CDI was extraordinary high (236/100,000), possibly due to the use of a test with a low positive predictive value[30] (ImmunoCard, Meridian Bioscience, Cincinnati, USA) and the inclusion of patients with a history of recent hospital admission[29].

Studies on patients with severe community-acquired diarrhoea requiring hospital admission who were subsequently diagnosed with CDI are not given in Table 1. It is estimated that these patients account for over 5% of all hospitalized CDI patients, emphasizing the importance of better guidelines for the diagnosis CA-CDI[14, 31]. Kuntz et al.[19] and Riley et al.[24] reported that 6% of the CA-CDI patients are treated for a recurrence. Bauer et al.[10] found a higher recurrence rate (29%); however, this study also included patients with a recent hospital admission. Although about 10% of the CA-CDI patients who are diagnosed by their GPs are hospitalized during the course of their disease[17, 23], CDI-related mortality rates in this group of patients are very low (≤3%)[23, 31, 32].

Patient and strain characteristics in CA-CDI

Patients with CA-CDI do not have the classic risk profile of patients who develop CDI in a healthcare facility. Only 32 to 88% used antibiotic therapy before their diarrhoea, and the mean age was below 65 years in all but one study (Table 1)[5]. Four studies that reported relatively low antibiotic usage might have suffered from patient recall bias[15, 27, 29, 32]. However, a large study by Dial et al. that used a drug prescription database also concluded that only 36% of the patients with CA-CDI used an antibiotic. Therefore, the variations in reported antibiotic use are probably attributable to the varying time intervals in which data were gathered and the differences in study populations. In a statewide surveillance study in Connecticut, 241 cases with confirmed CA-CDI

were reported by healthcare providers to the Department of Public Health. One-quarter of them had no underlying illness or hospitalization in the preceding year.[20] Similar results were seen in four other studies, where 16%, 26%, 35% and 40% had no antibiotic use or admission preceding their CA-CDI[23, 27, 32, 33]. Furthermore, severe CDI was reported among previously low risk populations, such as healthy individuals and pregnant women[4]. The emergence of CDI among pregnant women was only reported in two small studies of ten patients[4, 34], the majority of whom had a history of prior antibiotic therapy. Larger studies have not confirmed the emergence of CDI in pregnancy. The susceptibility to CDI of patients without traditional risk factors is not well understood. Proton pump inhibitors, which are used to treat reflux disease and peptic ulcers, were postulated to increase the vulnerability to *C. difficile*. Several studies reported discrepant results, and there is no consensus on whether this frequently used treatment predisposes to CDI[5, 31, 35, 36]. Identification of additional factors that increase vulnerability is therefore needed. The selection of an appropriate control group is essential for this purpose. Many recent studies compared hospitalized CDI patients with CDI patients from the community, which will not result in identification of new risk factors.

In order to explain the emergence of CA-CDI, new routes of transmission have been considered. A disease transmission model proposed by Otten et al.[37] mentioned four potential sources: the environment, contact with infected or colonized patients, contact with infected or colonized animals, and foodborne transmission. Increased exposure to one or more of these sources might explain the increase in the number of cases of diagnosed CA-CDI. However, as no outbreaks of CDI have been reported in the community, host factors that increase vulnerability might be of more importance in development of CDI than increased exposure to *C. difficile*.

A study of 57 patients with CA-CDI who were diagnosed by their GPs showed an association between CDI and contact with infants under 2 years of age in univariate analysis[23]. This association had not been found previously, possibly because it had not been looked for. The absence of a multivariate analysis implies that this association could have resulted from confounding. However, infants are known to be frequently colonized (approximately 40%) with toxigenic *C. difficile*[38]. These children rarely develop symptoms, and this is hypothesized to be attributable to the lack of a receptor for toxin A, but evidence for this hypothesis is lacking.

Information on the strains of *C. difficile* isolated from patients with CA-CDI is scarce and available from only a few small studies. The most frequently found PCR ribotypes were 078, 001 and 014[10, 39, 40]. These ribotypes are also among the most

prevalent in hospitals[41, 42]. Ribotype 027, however, was also found in smaller numbers than in hospitals.[39, 43] Strains such as ribotype 027, especially its spores, spread more easily within the hospital, because they can resist the hospital environment, cleaning, and disinfectants[44]. Variation in antibiotic prescriptions might account for the higher prevalence of type 027 in hospitals.

Clostridium difficile in animals and potential for transmission

CDI in animals was unknowingly described in 1968 when Small et al.[45] reported a case of fatal enteritis in laboratory hamsters after administration of antibiotics. Since then, hamsters have been used as animal models to prove the association of *C. difficile* with pseudomembraneous colitis in humans.[46] *C. difficile* has been isolated from almost all mammals[47, 48], including cows, horses[49], pigs[50], elephants[51], Kodiak bears[52] and non human primates[53], and also poultry[54] and ostriches[55]. In contrast to human medical research, where studies are mostly focused on the role of *C. difficile* in disease, many studies in animals concentrate on the presence of the bacterium in healthy animals. Investigations on the role of household pets as a possible reservoir of *C. difficile* showed that both healthy and diseased dogs and cats can shed spores of *C. difficile*[56, 57]. Riley et al.[57] sampled dogs and cats that were treated for a variety of reasons at two veterinary clinics, using selective solid and enrichment media, and found *C. difficile* in 39.5%. At both clinics, the environment became grossly contaminated with *C. difficile* as 40% and 75% of the sites were positive. Both toxigenic and non-toxigenic *C. difficile* isolates were recovered, but no overlap between animal and human isolates of *C. difficile* was found after typing[58]. In 2010, *C. difficile* colonization of pets and contamination of households was again evaluated by Weese et al.[59]. In 26 (31%) of the 84 households that were sampled, 14 (10%) of 139 dogs and three (21%) of 14 cats were positive for *C. difficile*. Again no overlap between canine strains and environmental isolates was seen after PCR ribotyping. In contrast to other studies, where the predominant ribotype in dogs and cats was the non-toxigenic 010, the most common ribotype in dogs and cats in this study was 001[60, 61]. This was also the most common ribotype among humans in the study area[59]. In fact, all toxigenic strains isolated from the pets in this study are known to be implicated in human CDI.

PCR ribotypes known to be involved in human CDI were also isolated from horses[60, 62]. Keel et al.[60] and Koene et al.[61] reported a diversity of ribotypes (>10 different types) in horses. Ribotype 015 was predominant according to Keel et al.,

whereas the Dutch study did not find a predominant ribotype. Songer et al.[63] reported a case of fatal typhlocolitis caused by ribotype 027 in a 14-year-old quarter horse. *C. difficile* seems to be a rare finding in healthy adult horses, as a low prevalence (0-1.2%) is reported for horses without signs of diarrhoea[64, 65]. The prevalence of the bacterium is higher in adult horses with diarrhoea and in foals, where it varies from 6% to 40%[64, 65].

Most of the published research on *C. difficile* in animals has been focused on production animals. The first large-scale study in food-producing animals was conducted in 1996 by Al Saif and Brazier[50], and although at least 100 animals from every animal species in the study, i.e. cattle, sheep, poultry, pigs and horses, from 40 different farms were sampled, *C. difficile* was isolated only rarely. The highest prevalence was found in poultry (1.6%) and the bacterium was not isolated from the pigs or cattle[50]. The age of the sampled animals was unknown, and because older age in animals is associated with a low *C. difficile* prevalence[66], the results of this study could be due to an age effect. Since the beginning of the 21st century the epidemiology of *C. difficile* in production animals has changed, because *C. difficile* is increasingly reported as a major cause of neonatal enteritis in piglets[67-69]. Even though the postulate of Koch was confirmed in two different studies in which piglets inoculated with *C. difficile* spores developed characteristic gross and microscopic signs of disease[68, 70], the role of the bacterium in disease in pigs is still questioned, since no association between diarrhoea and presence of the bacterium was found in a large Spanish study[71]. No clear correlation between disease and the presence of the bacterium was found in calves either[72, 73]. The ribotypes of isolates originating from cattle and pigs are much less diverse than those in dogs, horses, and humans.[60] The predominant PCR ribotype is 078, which accounted for 94% and 83% of the bovine and swine isolates in the study by Keel et al.[60] and for 100% of the isolates in a study by Keessen et al.[74]. In poultry, the association between enteritis and colonization with *C. difficile* is less well studied[54, 75, 76]. Zidaric et al. ribotyped 44 isolates from two separate flocks at one poultry farm. A wide variety of 12 different ribotypes was found, with none of them being predominant[54]. An overview of the predominant ribotypes of *C. difficile* in animal species is given in table 2.

Table 2. *Clostridium difficile* in animal species.

Animal species	Predominant Ribotype	Frequency N type/ total (%)	Study period	Reference
dogs	010	5/12 (42)	2007 (published)	Keel et al.[60]
	010	12/29 (41)	2009-2010	Koene et al.[61]
	001	4/14 (29)	2005-2006	Weese et al.[59]
	014	7/29(24)	2009-2010	Koene et al.[61]
cats	010	9/18 (50)	2009-2010	Koene et al.[61]
	039	5/18((28)	2009-2010	Koene et al.[61]
horses	015	6/20 (30)	2007 (published)	Keel et al.[60]
pigs	078	33/33 (100)	2008 (published)	Debast et al.[80]
	078	66/66 (100)	2009	Keessen et al.[74]
	078	93/144 (84)	2007 (published)	Keel et al.[60]
	078	7/9 (78)	2009-2010	Koene et al.[61]
	066	166/247 (67)	2009 (published)	Avbersek et al.[62]
	066	66/133 (50)	2008 (published)	Pirs et al.[111]
	SL011*	74/247 (30)	2009 (published)	Avbersek et al.[62]
	SL011*	31/133 (23)	2008 (published)	Pirs et al.[111]
	126	16/144 (11)	2007 (published)	Keel et al.[60]
	002	6/144 (4)	2007 (published)	Keel et al.[60]
	029	7/247 (3)	2009 (published)	Avbersek et al.[62]
cattle	078	31/33 (94)	2007 (published)	Keel et al.[60]
	078	31/33 (94)	2008 (published)	Hammitt et al.[112]
	012	5/6 (83)	2009-2010	Koene et al.[61]
	017	8/31 (26)	2004	Rodriguez-Palacios et al.[72]
	078	7/31 (23)	2004	Rodriguez-Palacios et al.[72]
	027	4/31 (13)	2004	Rodriguez-Palacios et al.[72]
	014	4/31 (13)	2004	Rodriguez-Palacios et al.[72]

* this type could not be identified.
Only when a ribotype was encountered in at least 4 animals per animal species, results were included in this table.

Although the issue of zoonotic transmission of *C. difficile* was raised more than 20 years ago, and the finding of overlapping ribotypes in animals and humans has stimulated research in this field, the question of whether zoonotic transmission occurs has not been answered. Circumstantial evidence that *C. difficile* strains from animals were infecting humans (or vice versa) has been reported several times in recent years[60, 77]. These studies have taken animal and human isolates and typed them by molecular methods, and have shown overlap between isolates in the two groups. For example, Arroyo et al.[77] looked at 133 isolates of *C. difficile* from dogs (n=92), horses (n=21) and humans (n=20), plus one each from a cat and a calf. Overall, 23 different ribotypes were identified. Of these, nine were identified from dogs, 12 from horses, seven from humans, and one each from the cat and calf. Although absolute numbers were small, 25% of the human isolates were indistinguishable

from animals isolates according to PCR ribotyping. Keel et al.[60] examined a similar number of isolates (n=144) and again showed similarities between horse, dog and human strains of *C. difficile* with PCR ribotyping, but not with strains from cattle or pigs. Other, more discriminatory, typing methods for *C. difficile,* such as multilocus variable-number tandem-repeat analysis or microarrays, also showed overlap between human and animal isolates[78-80]. Whether *C. difficile* strains in humans and animals are really identical should be determined by, for example, whole genome sequencing. The similarities seen in strains of human patients and different animal species do not automatically imply that interspecies transmission occurs. However, as living with an immunocompromised person is a risk factor for colonization with *C. difficile* for dogs[59], and the risk of *C. difficile* colonization of hospital visitation dogs is associated with close human contact[81], interspecies transmission is likely to occur.

In The Netherlands an overlap between the location of pig farms and the occurrence of human *C. difficile* ribotype 078 infections, which are increasing in prevalence, is observed[82]. The fact that infections with ribotype 078 in humans occurred in a younger population and were more frequently community-acquired than infections with ribotype 027 strains, together with the fact that 078 is the predominant ribotype in piglets, suggested a common source[82]. This common source is likely to be the environment. If infection rates in pig farms in the Netherlands are as high as those in the USA[67], it is likely that a large proportion of the Dutch population comes into contact with *C. difficile* spores every day, especially since the Netherlands has one of the highest population densities in the world. There is little evidence that other epidemic strains have zoonotic potential.

Environmental contamination

Because of its spore forming ability, *C. difficile* can survive in the environment for several months. The presence of *C. difficile* spores in hospitals is well established[83]. Also, gross contamination of farms such as pig facilities with *C. difficile* spores is commonplace. *C. difficile* could be isolated from the faeces of piglets 1 h after birth, presumably ingested from their environment. Within 2 days of birth, 100% of piglets had acquired *C. difficile* of the same molecular type that was found in sow faeces, sow teats, farrowing crates, and air on the farm[84]. There is evidence that vertical transmission does not occur in pigs[84]. Aerial dissemination of *C. difficile* on a pig farm has been shown to correlate with the activity of personnel within farrowing units[85], suggesting that staff might be at increased risk of ingesting airborne *C. difficile*

spores. Contamination of the pig farm environment was confirmed in another study where *C. difficile* prevalence in the environment increased from 0% to 61% of sites within a pig farrowing facility only 1 month after it has been occupied with pigs[86]. *C. difficile* spores and vegetative cells are shed into the immediate environment in the faeces of both scouring and non-scouring pigs, underscoring the importance of high carriage rates in apparently healthy piglets[84]. The carrier state is also emphasized in mouse studies that have demonstrated a marked increase in spore shedding when antibiotics are given to asymptomatic carrier mice. Subsequent spore-mediated transmission to immunosuppressed mice led to severe intestinal disease[87]. Another important consideration in relation to environmental contamination is effluent arising from piggeries. In Australia, piggery effluent is treated in anaerobic ponds to remove pathogens, and re-used to wash sheds or applied to agricultural land. *C. difficile* was shown to survive this process, with concentrations of viable *C. difficile* spores of greater than 200 CFU/mL (Squire and Riley, unpublished) posing a risk for infection of animals or contamination of agricultural produce.

Besides environmental contamination in the vicinity of colonized or infected humans and animals, *C. difficile* spores can be isolated from practically any environmental site, provided that the correct culture enrichment methods are employed[88]. A large study by Al Saif and Brazier[50] showed high rates of detection of *C. difficile* in soil and water samples in South Wales. Soil contained *C. difficile* in 21% of 104 samples, and 41% of the isolates produced toxin A. Water was positive in 88% of river samples, half of the sea, lake, and swimming pool samples, and 5.5% of the tap water samples. Overall, 85% of the isolates produced toxin A. In 2010, similar percentages were found in Slovenia[89], where 61% (42 of 69) of the river isolates was positive for *C. difficile*. Interestingly, 34 different types were found, more than half of which were also found in humans and animals. Ribotype 014, a common ribotype found in humans, was the most prevalent (16%). Although absolute counts of toxigenic *C. difficile* in water are low (1-5 CFU/100ml)[50], the infectious dose is unknown, and therefore so is the impact of the environment as a source of human or animal CDI.

C. difficile in food products

As *C. difficile* can be detected in live animals, foodborne transmission via meat is also considered to be a potential source of CA-CDI. Recently, a number of studies

have been published on the prevalence of *C. difficile* in (processed) meat, fish, and vegetables. These results are summarized in Table 3.

Remarkably, studies conducted in Europe persistently reported low prevalence rates, e.g. in up to 3% of meat samples[90-95], compared to the USA and Canada where *C. difficile* is generally reported at much higher rates, e.g. in up to 42% of meat samples[96-104]. Although high isolation frequencies are reported for *C. difficile* in meat, quantitative studies show that levels of contamination are generally low, with <100 CFU/g in chicken meat [101] and typically 20 to 240 spores/g in retail beef and pork[99]. Despite the low numbers, the spore forming nature of *C. difficile* and the heat tolerance of the spores[96] might facilitate foodborne transmission[101]. The majority of *C. difficile* isolates that have been recovered from food are toxigenic and therefore potentially pathogenic, with a clear overlap in types being found in human patients. PCR ribotypes 078 and 027 have not been isolated from meat samples in Europe, but are the main ribotypes found in food in North America (Table 3). However, this finding needs to be confirmed, because laboratory cross-contamination may have occurred in some studies[105]. If we exclude the study by Songer et al.[98], who found a high prevalence rate, the overall prevalence rate of *C. difficile* in meat samples in North America drops to 2%-20%, and more resembles the percentages found in Europe. Meat has been given most attention, and limited information is available on other food products. *C difficile* has been found in seafood and fish[50, 106], and also in vegetables[50, 107, 108] and environmental samples[50]. So far, the isolation of *C. difficile* from milk and milk products has not been reported, despite the presence of *C. difficile* in cattle faeces.

Whether the differences observed between countries, both in overall prevalence rates and in ribotypes, truly reflect geographical differences in occurrence, reflect temporal or seasonal differences in prevalent ribotypes or perhaps are caused by other factors is presently unknown and needs further investigation. Conceivably, the differences are affected by the use of different methodologies, although these do not seem to be related to distinct regions. Poor reproducibility with some methodologies has been shown, suggesting that present culture methods might be suboptimal for the detection of *C. difficile* in meat samples[97, 109]. Furthermore, the interpretation of findings is hampered by the use of different sampling methods. Validated methodologies for the sampling and isolation of *C. difficile* from food and environmental samples are urgently needed. The source of contamination with *C. difficile* in retail meats is also presently unknown. It may involve faecal or environmental contamination of carcasses, or contamination during processing by

shedding handlers[96, 98]. In addition, ante mortem deposition of (dormant) spores in the animal's muscle or other tissues has been suggested as a possible food contamination route[97, 109].

Although *C. difficile* is present in food for human consumption, and overlapping PCR ribotypes from animal and human sources have been reported[58, 77, 110], foodborne infection caused by *C. difficile* has never been confirmed. Further studies are required to provide relevant data on the sources, transmission routes, growth and survival of *C. difficile* in foods. Additionally, more information on the infective dose and more quantitative information on the level of contamination are needed to further measure the risks for humans associated with food-borne exposure to *C. difficile*.

Conclusion

C. difficile frequently causes mild, self limiting diarrhoea in the community. Only a minority of these patients seek medical attention. *C. difficile* is also found in animals, food products and the environment. To date, direct transmission from one of these sources to humans has not been proven, and there is little evidence that frequently found PCR ribotypes such as 001, 014 and 027 have a zoonotic source. We therefore believe that the overall epidemiology of human CDI is not driven by amplification in animals. However, because almost all PCR ribotypes are able to colonize or infect different hosts, and host-specific PCR ribotypes do not seem to occur, we assume that zoonotic transmission is possible. The emerging *C. difficile* type 078 in humans is epidemiologically linked to its presence in piglets, calves, and their environment, suggesting zoonotic transmission. Because this evidence is circumstantial, it needs to be determined whether patients at risk for CDI can truly be infected by these animals or their environment. The risk for infection of persons in close contact to these animals is likely to be small, although preliminary data indicate that colonization frequently occurs (Keessen et al, manuscript in preparation). The zoonotic potential of other frequently found pathogenic *C. difficile* ribotypes is probably very low. However, when new PCR ribotypes emerge, zoonotic transmission should always be considered.

Table 3. *Clostridium difficile* in food products.

	Country	Sample material	Npos samples/ Ntested samples (%)	Toxinogenic/ all isolates (%)	RT 078 or related strains* (%)	RT 027 or related strains** (%)	Other toxinogenic types (%)	Reference
Retail beef and veal								
North America	Canada	Ground meat	12/60 (20.0)	11/12 (91.2)	-	8/12 (66.7)	RT 077 (16.7) RT 014 (8.3)	Rodriguez-Palacios et al. (2007)[95]
	Canada	Ground beef and veal chops	13/214 (6.1)	12/15 (80.0)	-	4/15 (26.7)	RT 077 (20.0) RT 014 (13.3) NAP9 (6.7), C (6.7), H (6.7)	Rodriguez-Palacios et al. (2009)[96]
	USA	Cooked and uncooked beef	14/33 (42.4)	14/14 (100)	10/14 (71.4)	4/14 (28.6)	-	Songer et al. (2009)[97]
	Canada	Ground beef	14/115 (12.2)	14/14 (100)	12/14 (85.7)	1/14 (7.1)	Toxinotype IX (7.1)	Weese et al. (2009)[98]
	USA	Ground veal	4/50 (8.0)	3/4 (75.0)	NT	NT	NT	Houser et al. (2011)[103]
Europe	Sweden	Ground beef	2/82 (2.4)	2/2 (100)	NT	NT	NT	Von Abercron et al. (2009)[90]
	France	Ground beef	2/105 (1.9)	2/2 (100)	-	-	RT 012 (100)	Bouttier et al. (2010)[91]
	Austria	Ground beef	0/30 (0)	-	-	-	-	Jöbstl et al. (2010)[93]
		Ground beef/pork	3/70 (4.3)	1/3 (33.3)	-	-	RT 053 (33.3)	
	Netherlands	Beef/calf	0/164 (0)	-	-	-	-	De Boer et al. (2011)[94]
	Switzerland	Ground beef/pork	0/46 (0)	-	-	-	-	Hoffer et al. (2010)[92]
Retail pork								
North America	USA	Cooked and uncooked pork	19/46 (41.3)	19/19 (100)	13/19 (68.4)	6/19 (31.6)	-	Songer et al. (2009)[97]
	Canada	Ground pork	14/115 (12.2)	14/14 (100)	10/14 (71.4)	1/14 (7.1)	Toxinotype IX (7.1) Toxinotype 0 (7.1) Toxinotype III (7.1)	Weese et al. (2009)[98]
	Canada	Ground pork and pork chops	7/393 (1.8)	6/7 (85.7)	-	4/7 (57.1)	Toxinotype 0 (14.3), Toxinotype XXVI (14.3)	Metcalf et al. (2010)[99]
	USA	Pork and swabs	23/243 (9.5)	23/23 (100)	22/23 (95.6)	-	Toxinotype XI (4.3)	Harvey et al. (2011)[101]

	Country	Sample material	Npos samples/ Ntested samples (%)	Toxinogenic/ all isolates (%)	RT 078 or related strains* (%)	RT 027 or related strains** (%)	Other toxinogenic types (%)	Reference
Europe	Austria	Pork	0/27 (0)	-	-	-	-	Indra et al. (2009)[89]
	Austria	Ground beef/pork	3/70 (4.3)	1/3 (33.3)	-	-	RT 053 (33.3)	Jöbstl et al. (2010)[93]
	France	Pork sausage	0/59 (0)	-	-	-	-	Bouttier et al. (2010)[91]
	Switzerland	Ground beef/pork	0/46 (0)	-	-	-	-	Hoffer et al. (2010)[92]
	Netherlands	Beef/calf	0/63 (0)	-	-	-	-	De Boer et al. (2011)[94]
Poultry products								
North America	USA	Turkey	4/9 (44.4)	4/4 (100)	4/4 (100)	-	-	Songer et al. (2009)[97]
	Canada	Chicken meat	26/203 (12.8)	26/26 (100)	26/26 (100)	-	-	Weese et al. (2010)[100]
	USA	Chicken meat	4/32 (12.5)	7/7 (100)	7/7 (100)	-	-	Harvey et al. (2011)[101]
Europe	Austria	Chicken meat	0/6 (0)	-	-	-	-	Indra et al. (2009)[89]
	Netherlands	Chicken meat	7/257 (2.7)	4/7 (57.1)	-	-	RT 001 (14.3), RT 003 (28.6) RT 087 (14.3)	De Boer et al. (2011)[94]
Other food and feed products								
North America	Canada	Vegetables, divers	5/111 (4.5)	2/5 (40)	3/5 (60)	-	NAP4/ Toxinotype 0 (40)	Metcalf et al. (2010)[99]
	Canada	Seafood/fish	5/119 (4.8)	0/5 (0)	4/5 (80)	-	-	Metcalf et al. (2011)[105]
	Canada	Dog and cat feed	1/25 (4)	NT	NT	NT	NT	Weese et al. (2005) 113
Europe	UK	Raw vegetables	7/300 (2.3)	5/7 (71.4)	NT	NT	NT	al Saif and Brazier (1996)[49]
		Fish gut contents	0/107 (0)					
	UK	Ready-to-eat salads	3/40 (7.5)	3/3 (100)	-	-	RT017 (67) RT001 (330)	Bakri et al. (2010)[107]
	Austria	Raw milk	0/50	-	-	-	-	Jöbstl et al. (2010)[93]

* NAP07, Toxinotype V or related strains.
** NAP01, Toxinotype III, M31 or related strains.
NT: not tested.

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