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Clostridium difficile in foods and animals: history and measures to reduce exposure

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Abstract

Many articles have summarized the changing epidemiology of *Clostridium difficile* infections (CDI) in humans, but the emerging presence of *C. difficile* in foods and animals and possible measures to reduce human exposure to this important pathogen have been infrequently addressed. CDIs have traditionally been assumed to be restricted to health-care settings. However, recent molecular studies indicate that this is no longer the case; animals and foods might be involved in the changing epidemiology of CDIs in humans; and genome sequencing is disproving person-to-person transmission in hospitals. Although zoonotic and foodborne transmission have not been confirmed, it is evident that susceptible people can be inadvertently exposed to *C. difficile* from foods, animals, or their environment. Strains of epidemic clones present in humans are common in companion and food animals, raw meats, poultry products, vegetables, and ready-to-eat foods, including salads. In order to develop science-based prevention strategies, it is critical to understand how *C. difficile* reaches foods and humans. This review contextualizes the current understanding of CDIs in humans, animals, and foods. Based on available information, we propose a list of educational measures that could reduce the exposure of susceptible people to *C. difficile*. Enhanced educational efforts and behavior change targeting medical and non-medical personnel are needed.

Keywords: *Clostridium difficile*, community, foodborne, prevention, meat, vegetables, seasonality, refrigeration, superdormancy, cooking

Introduction – why is *Clostridium difficile* relevant today?

First associated with disease in humans in the mid-1970s, *Clostridium difficile* is a spore-forming bacterium that produces major toxins responsible for mild-to-severe forms of gastrointestinal infections in most mammals. Severe *C. difficile* infections (CDIs) in humans have steadily increased in hospitals, and alarmingly in the community, over the past three decades, especially among elderly over 65 years old (Freeman *et al.*, 2010). Because the life expectancy in humans and the proportion of elder citizens will rise globally (United-Nations, 2007), more CDIs are expected to occur in the future. Correspondingly, health-care costs associated with treatment are also expected to increase over time. Currently, the USA spends over $1.1 billion treating over half million CDIs every year.

To date, research has vastly focused on disease diagnosis, treatment, and control in hospital settings (Cohen *et al.*, 2010; Barbut *et al.*, 2011), but very little has been reported on prevention at the community level. Unlike in hospitals, younger individuals, pregnant women, and children have emerged as susceptible groups in the community since the mid-2000s (Barbut *et al.*,

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2011). In addition, individuals with inflammatory bowel diseases (IBD; i.e. Crohn's disease and ulcerative colitis), who mostly suffer life-long immune-mediated chronic recurrent gastroenteritis, have increasingly experienced more complications and mortality due to superimposed CDIs (Nylund *et al*., 2011). In IBD patients (more than 1.4 million in the USA), who are often treated as outpatients (in the community), CDI can be seen in as many as 10% of IBD patients seeking hospital medical attention. Infection without prior healthcare contact or antibiotic exposure is common in IBD patients. Currently, there are growing concerns that IBD flare ups can be due in part to CDIs. Although there were earlier indications that *C. difficile* could be contributing to IBD, traditionally patients were not screened for CDI, because earlier studies found no association with *C. difficile* (Goodhand *et al*., 2011). Treatment of IBD superimposed with CDI is becoming increasingly problematic, especially among adults with ulcerative colitis, and children who are increasingly likely to have concurrent CDIs (OR=11.42; 95% CI, 10.16–12.83) (Nylund *et al*., 2011).

Outside hospitals, it is known that certain environments, animals and foods are predictable sources of *C. difficile* (Gould and Limbago, 2010; Hensgens *et al.,* 2012), but this growing body of literature remains poorly communicated to health care professionals and the public in general. To date, no CDI cases have been confirmed to be of zoonotic or foodborne origin. Nevertheless, an increasing number of studies have shown that *C. difficile* with the toxins and potential to cause disease can often be found in animals, recreational waters, and raw and ready-to-eat foods, in variable frequencies (i.e. 0–66%) (Rodriguez-Palacios *et al*., 2012; *C. difficile* capable of producing toxins (which cause intestinal lesions) has been isolated from at least 70.3% (26/37) of food groups (representing independent studies and over 3519 food items) tested with enrichment methods in Europe and North America (see Rodriguez-Palacios *et al*., 2012 for a review). The discrepancy between studies that isolate *C. difficile* and reports with 0% prevalence can be due to culture method choice (i.e., use of selective enrichment), and the increasingly recognized effect of spore age, superdormancy, thermoresistance, and sample refrigeration on our ability to detect *C. difficile* (Rodriguez-Palacios and LeJeune, 2011; Thitaram *et al*., 2011, Kho, 2012, Limbago *et al*., 2012).

Although it is difficult to predict if a given food item, animal, or water source will have sufficient *C. difficile* (if any) to make someone sick, it is more feasible to predict who are the most susceptible individuals so as to educate and protect them. Although *C. difficile* may be introduced into health-care centers (hospitals/nursing homes) via the hands or clothing of new patients (both symptomatic and asymptomatic), visitors, or healthcare workers themselves, next generation whole genome sequencing has shown that patients during CDI outbreaks are getting ill with *C. difficile* strains that cannot be explained by person-to-person transmission alone (Eyre *et al*., 2012). Strains affecting people appear to be coming from outside healthcare centers. A recent study of *C. difficile* in the skin of people in two community settings in the USA and Ireland showed that it is more likely to be exposed to foods, which have the potential to carry *C. difficile* (up to 42%) than to be exposed to animals, recreational waters, hospitals settings, or to *C. difficile* on unwashed hands (<0.7%) (Rodriguez-Palacios *et al*., unpublished data). Even the general ward environment of community hospitals has relatively low prevalence of *C difficile* (2.4%) (Faires *et al*., 2012) compared to some food groups (Rodriguez-Palacios *et al*., 2012).

History and disease burden

Financial and social costs

CDI is a costly disease in most countries. In the USA, estimates indicate that there are about 500,000 CDIs every year, which result in $1.1–3.2 billion in health care costs every year (O'Brien *et al*., 2007). On average, each new CDI infection costs $3000–5000, whereas recurrent infections (more difficult to treat) cost $13,000–18,000 (Dubberke and Wertheimer, 2009; Ghantoji *et al*., 2010). Similar high treatment costs have been documented in Europe (Wilcox *et al*., 1996; Ghantoji *et al*., 2010).

With an increase in the number of human infections, there has also been an increase in social concerns. Over time the incidence of severe disease has increased with more patients requiring surgical removal of the inflamed colon (one in every ten CDI cases – 10%), higher mortality rates, and concern about increased liability (Pepin *et al*., 2005; Sailhamer *et al*., 2009; Marler, 2010). The effects of such medical consequences at the individual and family level are difficult to quantify. Infection control and surveillance initiatives have thus been reinforced in hospitals to reduce the incidence of *C. difficile* (Pepin *et al*., 2004; Barbut *et al*., 2011) with variable success.

Given that *C. difficile* also infects animals, the disease can have a financial impact on companion animals and livestock also. Financial loss estimates associated with CDI in animals are not available. However, in horses and other companion animals where *C. difficile* causes enteric disease, the costs associated with veterinary medical treatment are high (several thousand dollars in North America) and generally assumed by the owners. In livestock production, no estimates are yet available either, although there is evidence that *C. difficile* causes disease and possibly growth delays in production animals (Songer, 2004; Kiss and Bilkei, 2005).

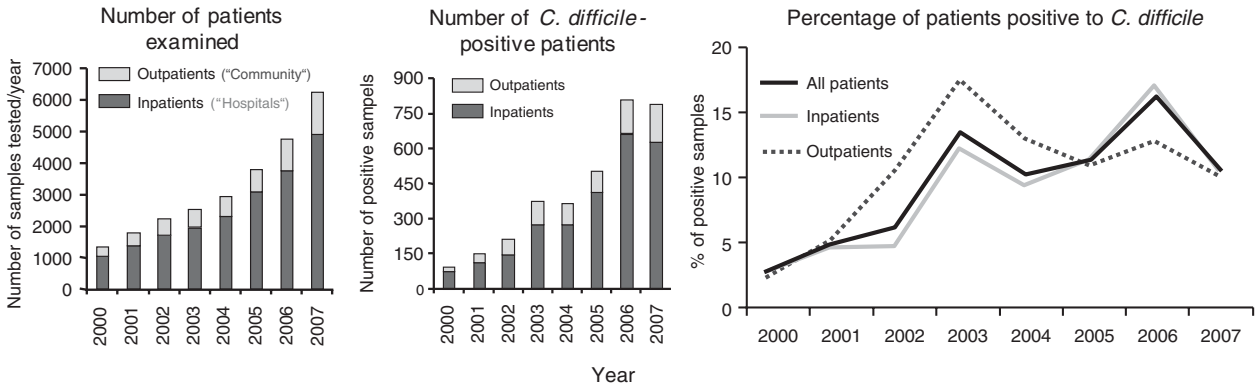

Fig. 1. Paralell increase in hospitals and the community. *C. difficile* toxins in fecal samples from patients visiting 40 hospitals and over 2000 physicians in southern Germany. (Reproduced with permission from Borgmann *et al*. 2008; Copyright Eurosurveillance).

Early history of *C. difficile* as a gut pathogen

C. difficile is a spore-forming anaerobic bacterium that was first isolated from stools of healthy infants in 1935 (Bartlett, 2008). Although these first bacterial isolates were fatal to hamsters, no attention was directed to the health risks of *C. difficile* in adults until decades later; *C. difficile* was deemed normal in the gut of children. In 1962, the same bacterium was isolated from localized infections (e.g. wounds and abscesses) in adults (Smith and King, 1962). Although these isolates were also fatal to hamsters, the authors concluded that *C. difficile* was not pathogenic for man.

Only in the late-1970s, additional studies in humans and hamsters confirmed *C. difficile* as the cause of a severe form of colitis in adults known since the 1890s as pseudomembranous colitis (PMC) (Bartlett, 2008). In PMC, marked inflammation and cellular debris accumulate over the intestinal surface giving the appearance of a pseudomembrane. Microscopically, PMC was characterized by exuberant inflammatory plaques formed on the surface of the colon protruding from the intestinal wall (Price and Davies, 1977). In humans, the cause of PMC was unclear for almost 80 years, until the 1970s when the administration of antibiotics, especially clindamycin and lincomycin, was linked to PMC (Tedesco *et al*., 1974). Initially researchers thought that PMC resulted from a viral infection and the concurrent use of antibiotics (Steer, 1975), but finally *C. difficile* was identified as the microbial cause secondarily linked to antibiotic use; which disrupts the gut flora favoring the opportunistic proliferation of *C. difficile* (Bartlett *et al*., 1978; George *et al*., 1978; Larson *et al*., 1978).

Currently, PMC is almost always (>95%) linked to *C. difficile* (Hurley and Nguyen, 2002), but not all CDIs result in PMC. In animals, a similar form of inflammation has been reported in a small fraction of piglets infected experimentally with *C. difficile*. In other animals (e.g. mice, hamsters, horses and calves), various forms of colitis have been described, from mild in most species to severe and fulminant in some horses (Colitis X, first case report was linked to an emerging hyper-virulent *C. difficile* PCR ribotype 027/NAP1 strain known to be highly problematic in humans) (Songer *et al*., 2009).

During the last decade, the severity of the CDI, including PMC, has increased in populations of children who were previously rarely affected. Today the growing epidemic and the more frequent lack of response to conventional therapies have raised the awareness of CDI to the point where it is increasingly recognized as a global public health challenge, often surpassing the importance of methicillin-resistant *Staphylococcus aureus* infections (Lessa *et al*., 2012).

C. difficile in hospitals and risk factors

CDIs were first seen as sporadic cases in humans, particularly in hospitals, but in the 1990s, the frequency increased (>2-fold) as highlighted in an article entitled *C. difficile*: a pathogen of the nineties (Riley, 1998). The problem has been especially notorious in developed nations (Pepin *et al*., 2004), and continues to extend into the 2000s; now it has been documented in community settings (Fig. 1) (Borgmann *et al*., 2008). Controlling for confounding variables, it is known that such increase is not due to reporting bias (Burckhardt *et al*., 2008). Other studies have also shown the remarkable parallel between the increased trend of disease in hospitals and the community (Noren *et al*., 2004). However, the incidence of CDI is much lower (1300-fold) in the community, compared to hospitals, due in part to a lower (37-fold) occurrence of antimicrobial consumption (Noren *et al*., 2004). Compared to other drugs, mortality data associated with drug consumption in the USA showed that among diseases with significant drug-related etiologies, *C. difficile* enterocolitis primarily associated with antimicrobials had the largest percentage increase in total mentions, with a 203% rise between 1999 and 2003 (Wysowski, 2007). Today, the increased resistance to

Table 1. Risk factors for CDIs

- Antimicrobials/antacids increase risk with combined use or long treatments
- Elderly (over 65 years old) are more likely to become ill
- Colon diseases; i.e. IBD or colorectal cancer
- Debilitating illness; cancer or immune-suppressive conditions/medications
- Abdominal surgery or gastrointestinal procedure
- Having had CDI already
- Current or past hospitalization or contact with CDI patients
- Living in a nursing home/long-term care facility

many antimicrobials, especially fluoroquinolones has become an emerging global health issue (Spigaglia *et al.*, 2008; Ashiru-Oredope *et al.*, 2012). Among cases with antimicrobial-associated diarrhea, CDIs account for about 25–30% of all cases. For decades, antimicrobial consumption has been the main predisposing factor for CDI.

Elderly over 65 years old have been always more susceptible to infections (Pepin *et al.*, 2004). Regarding the source of infection, by the end of the 1990s, humans were considered to be the sole reservoirs for infection to other humans (Kaatz *et al.*, 1988). However, studies from the 1980–1990s outside hospitals in the UK demonstrated that *C. difficile* was present in water bodies in connection with urban settings, soils, root vegetables, and household pets (Borriello *et al.*, 1983b; al Saif and Brazier, 1996). The potential for animal–human and foodborne transmission was then highlighted. Although genetic testing of recovered strains determined that most isolates were capable of producing toxins (necessary for intestinal disease), no molecular typing was reported to determine if they were the same strains affecting humans.

The increasing number of cases inside hospitals maintained the attention on human-to-human transmission, mediated by environmental contamination of the hospital wards and health care personnel (Kaatz *et al.*, 1988). Infections originating in the community, where patients acquire CDI outside hospitals, were considered infrequent and received no attention for disease prevention. No connection or differentiation was acknowledged between community- and hospital-acquired CDI until the last decade. Currently, there are more defined criteria to classify new CDIs as community- or hospital-associated cases based on the site of acquisition or onset of clinical signs (Kuijper and van Dissel, 2008). A similar differentiation has importantly been used in epidemiological studies in veterinary hospitals since the mid-2000s, especially in Canada (Weese *et al.*, 2006).

Despite the rapid recognition of increased virulence and ecological aspects of *C. difficile*, medical textbooks continue treating *C. difficile* as the traditional medical condition acquired only in people exposed to hospitals or long-term health care settings with little attention focused on ecology and prevention. In veterinary medicine, *C. difficile* is still invariably reported in reference medicine books as an organism associated with clinical disease in animals, with limited emphasis on public health. Most veterinary literature largely remains as review articles.

Although several risk factors for CDI have been identified for humans over the past decades (see Table 1), it is noteworthy to highlight that the ages at which people get CDI (and possible exposure to other unknown risk factors) are parallel but significantly different in hospitals compared to the community. Younger individuals (although less likely to suffer CDI than the elderly) are comparatively more often affected in the community (Hirshon *et al.*, 2011). The distribution of ages of CDI patients depicted in Fig. 2 highlights that in hospitals most inpatients are significantly older when compared to the age of outpatients treated by the same center during the same period. Regarding the traditionally known risk factors (Table 1), the following excerpt illustrates that disease trends are changing: 36% of patients had no history of antibiotic use within 3 months before symptom onset, and 25% had no underlying medical condition or recent hospital admission and, moreover, were younger than 45 (Kuijper and van Dissel, 2008; Hirshon *et al.*, 2011). CDI can no longer be considered a disease exclusively acquired in hospitals.

Age is a very important risk factor for disease. Although cases can occur in children elderly are more prone to CDI. Recent studies in long-term care facilities showed that over 50% of patients develop CDI beyond the fourth week after hospital discharge, much longer than for acute-health care settings, highlighting the importance of long-term disease prevention (Pawar *et al.*, 2012). In the community, cancer patients, Crohn's and ulcerative colitis patients, and other individuals receiving immunosuppressants and antibiotics are at risk for CDI. In humans, between 20 and 27% of CDIs that require hospital-level medical treatment are acquired in the community. Based on risk factors known (Table 1), prevention measures could be focused on susceptible individuals.

Theory of person-to-person transmission disproved

The concept of hospital clonality, long suspected to be caused by a single highly infectious strain with clonal dissemination within hospital wards based on fingerprinting qualitative typing techniques, has been increasingly questioned for CDI. Using the latest portable sequencing technology, early in 2012, a whole-genome sequencing study of *C. difficile* isolates from cases assigned to three hospital outbreaks in UK demonstrated that most consecutive CDIs were due to different strains and so (in their own words) refuted the theory of person-to-person transmission to explain the increase incidence of CDI within hospital wards (Eyre *et al.*, 2012). For one cluster of CDI involving three people, over 4 days in the same

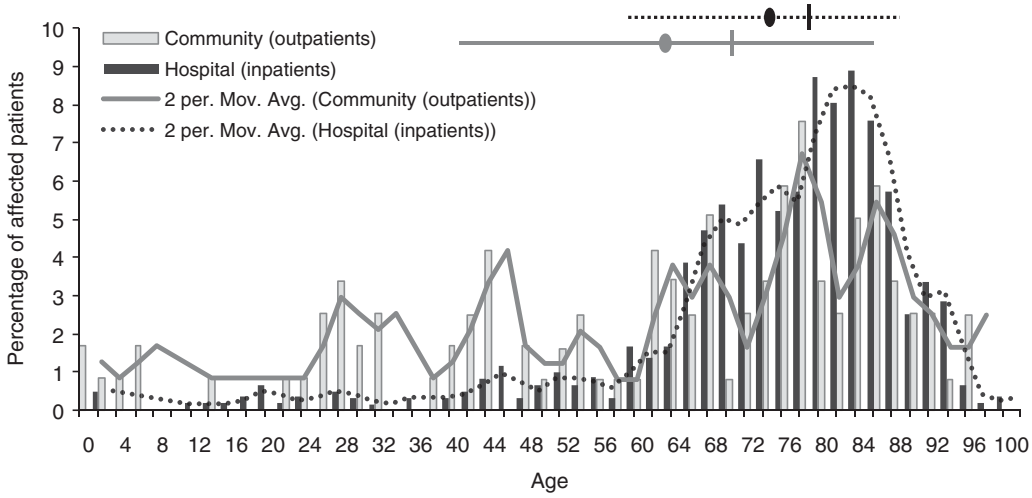

Fig. 2. Affected people in the community are younger than affected people in hospitals. Percentage of humans with CDI in hospitals and the community, Germany 2006. Total number of patients, $n = 714$. Mov. Avg.=moving average. Horizontal bars represent average (oval)±S.D., and the medians (vertical ticks). (Data courtesy of Dr S. Borgmann *et al.*)

ward the authors concluded that next generation sequencing refutes transmission between suspected linked cases and that isolates of the same strain type are not necessarily linked by person-to-person transmission. Data from this and two other clusters demonstrated that person-to-person transmission within hospitals is not as exclusively high as previously thought. *Clostridium difficile* strains appear to be introduced to hospitals by incoming patients (and possibly foods/visitation animals) more commonly than earlier suspected.

CDIs and toxin types

Numerous reviews describing the biology and epidemiological changes of CDI in humans are available. In animals, similar papers have been published since the first review describing *C. difficile* as an emerging pathogen in food animals in 2004 (Songer, 2004, 2010; Gould and Limbago 2010; Weese, 2010; Hensgens *et al.*, 2012; Rodriguez-Palacios *et al.*, 2012). All reviews indicate that animals and foods are reservoirs of *C. difficile* strains that produce toxins. From experimental studies in animals, it is possible to say that CDI occurs only when *C. difficile* opportunistically proliferates in the intestinal tract of its host (animal or human) and produces its toxins that are deleterious to the intestinal wall. In this context, several factors are needed including: (1) the ingestion of *C. difficile* spores and the persistence of *C. difficile* in the intestinal tract, (2) the proliferation of *C. difficile* and the production of toxins in the gut, and (3) an immunologically susceptible host with disarranged gut flora.

Soon after its identification as a pathogen it was determined that the pathogenicity of *C. difficile*, was mediated via two similar, but structurally and immunologically distinct, virulence factors: Toxins A and B (Bongaerts and Lyerly, 1997). Once in the cell, these toxins affect glycosylate Rho GTPase, a key enzyme in signaling pathways regulating actin polymerization. The net effect is a disruption of normal cytoskeletal architecture leading to cell death followed by local and systemic inflammatory reactions (Mazuski *et al.*, 1998; Hamm *et al.*, 2006; Sun *et al.*, 2010; Modi *et al.*, 2011). Another toxin, called binary toxin, present in a fraction of *C. difficile* strains may also contribute to disease (Geric *et al.*, 2004, 2006; Terhes *et al.*, 2004; Stare *et al.*, 2007; Schwan *et al.*, 2009; Sun *et al.*, 2010).

Almost always, strains capable of causing disease carry both the toxins A and B, denoted A^+B^+. However, since 1999, naturally occurring *C. difficile* mutant strains, lackng toxin A (A^-B^+), have caused major outbreaks in hospitals internationally (al-Barrak *et al.*, 1999; Loo *et al.*, 2005; Lyras *et al.*, 2009; Kuehne *et al.*, 2010; Sun *et al.*, 2010). In the past, A^-B^+ strains were uncommon (<5%); nowadays, those strains are problematic and predominant (>30%) in certain regions (Kim *et al.*, 2008; Shin *et al.*, 2008a, b). Since the strains that do not produce either toxin A or toxin B (A^-B^-) are non-pathogenic (Bongaerts and Lyerly 1997; Rupnik *et al.*, 2005), there has been interest in therapeutic microbiology using these strains as probiotics to prevent colonization in susceptible hospitalized people. Experiments in various animal species support the potential benefit. Noteworthy, non-toxigenic strains (A^-B^-) are comparatively more common in mature food animals than in people and foods, but A^-B+ in foods appear to be less common (Bakri *et al.*, 2009; Hensgens *et al.*, 2012).

In addition to toxin-based studies, the advent of genomics and systems biology have spurred the increasing documentation of other possible virulence factors since the early 2000s. Therapeutically, such knowledge still has not yet resulted in new effective measures to treat CDI in hospitals. Immunologically, most high risk or severely ill patients have low levels of antibodies against

Table 2. Reported sources of *C. difficile* outside hospitals (in the community)

- Contaminated foods (difficult to predict/notice; variable; *C. difficile* has been found in food with good organoleptic quality).
  The prevalence ranges from 0 to 42%, although most studies have reported prevalences below 7%
- Healthy animals shedding *C. difficile* (difficult to predict/notice; animals look healthy).
  The prevalence can be as low as 0% in adult healthy animals, to 100% in young piglets.
- Diseased animals suffering CDI can shed *C. difficile* in feces (predictable, possible but unclear role in human disease; animal-to-animal transmission has been partly documented)
- Animal waste (occupational risk is possible but unknown; occupational risk has been documented for laboratory personnel taking antimicrobials and working with *C. difficile* carrying specimens).
- Contaminated environment (difficult to predict/notice unless obvious influence of potential source of *C. difficile*; for instance, sewage from human/animal facilities; *C. difficile* has been found in recreational waters).

toxins A and B, while healthy individuals appear to have higher titers (Kelly and Kyne, 2011). Therapeutic interest is now in the use of antibody supplementation to currently approved therapies against CDI, largely based on antimicrobials against *C. difficile*, i.e. metronidazole and vancomycin, and DNA based vaccines against toxins A and B (Jin *et al.*, 2013). However, increased resistance to such antimicrobials is also increasing (Sinh *et al.*, 2011). Patented human monoclonal antibody technology is in phase clinical trials. Thus far, the therapeutic benefit seems to be present when antibodies are supplemented in mild-moderate cases; but the response is poor in severe CDIs. Epidemiological and experimental data indicate that immune susceptibility and bacterial flora disarrangements are major factors for CDI. Aside from reinforcing hand washing, little has been done on actively involving the communities at risk to prevent exposure to *C. difficile*.

Increased antimicrobial resistance and ability to produce toxins

Compared to isolates from before 2000, current *C. difficile* isolates affecting people are more resistant to antibiotics (Warny *et al.*, 2005; Sinh *et al.*, 2011). Further, some strains arguably can produce up to 16-to-20 times more toxins (A or B) *in vitro* compared to regular strains (Loo *et al.*, 2005; Warny *et al.*, 2005). Therein, those strains increasingly isolated in current times are often referred to as 'hyper-virulent' strains (Mulvey *et al.*, 2010). The most widely factor associated with the increased ability to produce toxins *in vitro* is the presence of a genetic mutation in a gene (*tcdC*) that normally down-regulates the genes responsible for the production of toxins A and B. In vivo, the association of *tcdC* polymorphisms with disease severity is less clear. Other virulence factors such as antibiotic-induced adherence to intestinal cells (Deneve *et al.*, 2008) and strain-dependent systemic toxin pathogenicity are possibly contributing features (Lanis *et al.*, 2012). Of public health relevance, hyper-virulent strains, associated with severe disease in humans, have been increasingly isolated from food animals and foods since 2006 (Rodriguez-Palacios *et al.*, 2007a; Hensgens *et al.*, 2012).

C. difficile recurrences are increasingly common

Following recovery from a CDI, reinfections in the same individual and treatment failure are occurring with more frequency. Recurrences after treatment of CDI with metronidazole (first drug of choice) have increased from 7% before the year 2000 to 29% thereafter (Kelly and LaMont, 2008; Sinh *et al.*, 2011). Although not everyone suffers reinfections, some individuals are overly sensitive. The reasons for such susceptibility are currently under investigation. Low antibody titers effective against the *C. difficile* toxins (Wilcox, 2004), and disrupted intestinal flora due to antimicrobials (Rupnik *et al.*, 2009) are among the factors that enhance susceptibility to reinfections. Increasingly, the administration of proton pump inhibitors (widely prescribed antacid) confers more risk for recurrence compared to other classes of antacids (Linsky *et al.*, 2010). More common since the year 2000, reported rates of reinfections have varied between 15 and 30%, with recurrences commonly seen among elderly (Kelly and LaMont, 2008).

The more recurrences a person has, the more likely he/she is to have a recurrence again. The risk of recurrence goes from about 20% after the initial CDI episode to about 40 and 60% after the first and two-or-more recurrences, respectively (McFarland, 2008). In at least 10% of cases, subsequent infections are caused by a new *C. difficile* strain that is molecularly different from that of the first CDI episode (Wilcox *et al.*, 1998; Noren *et al.*, 2004; Hell *et al.*, 2011). As more discriminatory typing methods become available (e.g., multiple-locus variable number tandem repeat analysis, MLVA; or next generation sequencing) (Marsh *et al.*, 2011; Eyre *et al.*, 2012), it is likely that more recurrences will be recognized to be indeed due to different strains, and not due to persistent infections. Currently, reinfection with different strains indicates that there are unrecognized sources of *C. difficile* in the community that serve as the source of infection for convalescent people following hospital discharge. Numerous studies have shown that animals, foods, and recreational environments can be sources of *C. difficile* strains similar or identical to those causing diseases in humans (Janezic *et al.*, 2012). Table 2 summarizes reported sources of *C. difficile* in the community.

Animals, the environment, and foods

In animals, the first studies reporting the isolation of *C. difficile* from companion animals and pigs were published in the 1980s. However, it was early in the 2000s when the association with enteric disease in animals was confirmed. Later, molecular fingerprinting comparing *C. difficile* isolates from companion animals and humans indicated for the first time the potential for identical strains to share human and animal habitats (Arroyo *et al.*, 2005). As an environmentally stable microorganism, transmission from animals to humans may occur via exposure to contaminated environments.

Although the possibility of animals being reservoirs of *C. difficile* relevant for CDI had been suggested for years, it was not until the mid-2000 that molecular evidence became stronger while studying food animals. A large microbiological survey conducted in dairy calves documented the etiological role of *C. difficile* in bovine neonatal diarrhea in ill calves after controlling for other pathogens; and the presence of epidemic human strains of international relevance (PCR ribotypes 078, 027, 014, and 017) in healthy calves (Rodriguez-Palacios *et al.*, 2006; Rupnik, 2007). Subsequently, *C. difficile* was recovered from ground meats (Rodriguez-Palacios *et al.*, 2007a), which appears to have a reproducible seasonal pattern that matches that of bovine, swine, and human CDIs in North America (Rodriguez-Palacios *et al.*, 2009) (see seasonality section below). Noteworthy is to mention that depending on the environment, not all animals carry *C. difficile* (Bandelj *et al.*, 2011; Rodriguez-Palacios, 2011). Predicting which animals are carriers is becoming less challenging as knowledge increases. This is important for prevention given growing indications of potential zoonotic transmission for some *C. difficile* strains, namely PCR ribotype 078; see Hensgens *et al.*, 2012 for a review on behalf of the European Society of Clinical Microbiology and Infectious Diseases Study Group for *C. difficile*.

Companion animals – household pets

In modern times, especially in urban areas, pets are an integral part of the family, sharing human lifestyles, bedrooms, and beds. Recent estimates indicate that between 14 and 62% of pet owners allow dogs and cats on their beds (Chomel and Sun, 2011; Montgomery *et al.*, 2011). Although dogs and cats have been shown to carry toxigenic strains of *C. difficile* in their feces since the 1980s (Borriello *et al.*, 1983b), the current striking genetic similarity between isolates from animals and humans indicate that zoonosis may be occurring (Lefebvre *et al.*, 2006). Most companion animals that harbor *C. difficile* do so asymptomatically (Weese *et al.*, 2010a). However, if risk factors are prevalent, that parallel those of humans (antimicrobial administration), dogs and cats may develop diarrhea (Weese *et al.*, 2001). No PMC or *C. difficile* bacteremia has being documented in dogs or cats.

Screening studies indicated that up to 10% of household pets may carry *C. difficile*, representing a risk for owners (Weese *et al.*, 2010a). Although no direct transmissibility from pets to humans has been documented, the presence of virulent strains of *C. difficile* (including PCR ribotype 027) in 'therapy' dogs indicate that in hospitals, visitation animals might carry strains within and outside health-care facilities (Lefebvre *et al.*, 2006). Pets owned by an immune-compromised person are more likely to be colonized by *C. difficile* (Weese *et al.*, 2010a). However, in one study that examined the zoonotic risk, the strains isolated from dogs and households were different (Weese *et al.*, 2010a) another indication against direct-contact transmission. Although the authors concluded that dogs were not a significant source of household *C. difficile* contamination, all isolates from dogs were indistinguishable from historical isolates recovered from ill humans in the same geographical region, including emerging PCR ribotype 027. Therefore, it is advisable to prevent close contact between susceptible people and pets with diarrhea. It is also important to highlight that inadvertent infections with *C. difficile* (or other enteric human pathogens) in healthy-looking pets could occur in association with the consumption of raw pet foods (Weese *et al.*, 2005; Finley *et al.*, 2006). Avoiding the inclusion of raw meats in pet diets is always a good practice to reduce the risk of transmission of *C. difficile* and other zoonotic pathogens, especially if high-risk individuals are in the household.

In veterinary hospitals, outbreaks of severe diarrhea associated with *C. difficile* have been reported in small animal clinics (Weese and Armstrong, 2003). Therefore, pets may become inadvertent carriers of *C. difficile* spores following routine veterinary visits or hospitalization. To date no studies have assessed the potential of dogs and cats to be vehicles of *C. difficile* strains out of veterinary hospitals and within food or livestock production systems.

Companion animals – horses

C. difficile has also been studied in horses since the mid-1980s (Ehrich *et al.*, 1984). Today, it is known that in the community up to 7% of healthy horses can carry *C. difficile* (Medina-Torres *et al.*, 2011), but the proportion of animals shedding the pathogen varies across studies as it depends on culture methods, the animals' age, and management conditions. Adult horses are less likely to carry the bacterium compared to neonatal foals. Overall, between 2 and 30% of horses were found to be carrying spores at any given time without showing signs of disease (Baverud *et al.*, 2003). However, like other species, horses can also develop diarrhea and forms of serious colitis (Weese *et al.*, 2006; Songer *et al.*, 2009). As in humans, antimicrobials increase the risk of horses being

affected with CDI (Weese *et al.*, 2006). In young foals, antimicrobials are also a predisposing factor (Arroyo *et al.*, 2004). Co-infection with *C. perfringens* may explain enterocolitis in some foals (Uzal *et al.*, 2011). In equine hospitals, strict isolation and infection control measures are thus widely recommended to avoid outbreaks (Baverud, 2004).

Some ethnic societies by tradition still rely on horse power to work agricultural lands to produce foods, especially fresh produce (Lengacher *et al.*, 2011). In many regions, regulated production and slaughter of horse meat is allowed to sustain, at least partially, local economies and traditions (USDA, 1997). Since horse manure can contain *C. difficile* spores for years (Baverud *et al.*, 2003) its use as traditional organic fertilizer highlights the risk for fresh produce contamination (Pell, 1997). To date, there are no studies addressing the role of horses in food and environmental health and safety associated with *C. difficile*. Nevertheless, farmers and animal handlers should be aware of the risk of finding *C. difficile* on horse manure and the potential for dissemination to susceptible members of the family or the community.

Food animals – pigs

As in companion animals, *C. difficile* was also isolated from pigs in the early 1980s (Jones and Hunter, 1983). Since then, over 60 published studies have served to now recognize *C. difficile* as an enteric pathogen in this domesticated species. Among pigs, young piglets have the highest risk for disease development (Post *et al.*, 2002). For this reason, pigs have been increasingly used as models to study the pathogenesis of this disease (Keel and Songer, 2007, 2011; Steele *et al.*, 2010; Scaria *et al.*, 2011). Mortality and morbidity rates in pigs are largely uncertain, but some estimates indicate that up to 100% of litters and individual piglets can be affected in infected farrowing facilities (Songer, 2004). In non-fatal cases, weaning weights of diseased pigs can be 10% below the expected average weight (Songer, 2004). In older animals, there is one report of an association between *C. difficile* and increased mortality in sows that received antimicrobial treatment (Kiss and Bilkei, 2005).

During processing, the isolation of *C. difficile* from healthy pigs close to the harvest time, and from processed carcasses (<2.5%) support the potential for food contamination (Norman *et al.*, 2009; Weese *et al.*, 2011, Susick *et al.*, 2012). Recent isolation of *C. difficile* from mesenteric lymph nodes at harvest (<1%) indicates that pathogen dissemination from the gut to muscles tissues via the circulatory and lymphatic system is possible (Susick *et al.*, 2012).

Swine-derived *C. difficile* isolates have garnered the greatest attention from public health personnel because PCR ribotype 078 – the increasingly documented emerging human strain in the community – is the major strain among porcine isolates. PCR ribotype 078 isolates have accounted for up to 80% of all swine isolates in most studies involving pigs in North America and Europe (Keel *et al.*, 2007; Debast *et al.*, 2009; Songer *et al.*, 2009). In humans, the same strain has increased its association with human disease by at least 6-fold from 2000 to 2008 (Goorhuis *et al.*, 2008). Regarding the type of production system, no differences have been found between the prevalence of *C. difficile* in organic and conventional swine operations (Keessen *et al.*, 2011), or between conventional and antibiotic-free operations (Susick *et al.*, 2012).

Food animals – cattle

Until recently little attention was directed to the study of *C. difficile* in ruminants. The first published report described *C. difficile* in veal calves with diarrhea in 2002 (Porter *et al.*, 2002). The first published study quantifying the impact of *C. difficile* in the bovine industry determined in 2004 the role of the pathogen as a cause of diarrhea in young calves, the effect of seasonality, and its implications for public health (Rodriguez-Palacios *et al.*, 2006). In that study, a case-control study of calves <28 days of age – from 102 dairy farms in Canada – showed that significantly more calves with diarrhea were positive for *C. difficile* toxins compared to the control group, suggesting an association of *C. difficile* with intestinal disease. In further experimental studies, the same group could not induce disease when calves fed colostrums were given orally high numbers of toxigenic *C. difficile* (Rodriguez-Palacios *et al.*, 2007a, b). Subsequent studies in calf ranches have supported the association of intestinal lesions with *C. difficile* (Hammitt *et al.*, 2008). Colonization of neonatal calves infected under natural conditions was detected within 24 h of birth and lasted for at least 6 days after detection, indicating that calves were indeed amplifiers of toxigenic *C. difficile* (Rodriguez-Palacios *et al.*, 2007a). Histological lesions were mild and restricted to ileum and colon. In veal calves, the rate of *C. difficile* shedding and associated diarrhea increases as animals are treated with antibiotics upon entry to finishing operations (Costa *et al.*, 2011). Strain clonal diversity and shedding prevalence in young farm animals decrease with age (Rodriguez-Palacios *et al.*, 2006; Zidaric *et al.*, 2012).

In older cattle, *C. difficile* shedding decreased over time during the finishing period and was not affected by the administration of antimicrobials (Rodriguez-Palacios *et al.*, 2011b). At the time of harvest, *C. difficile* can be found in healthy feedlot steers and culled dairy cattle, highlighting the risk for carcass and food contamination (Rodriguez-Palacios *et al.*, 2011a, b; Thitaram *et al.*, 2011). In Belgium, the frequency of shedding at slaughter was

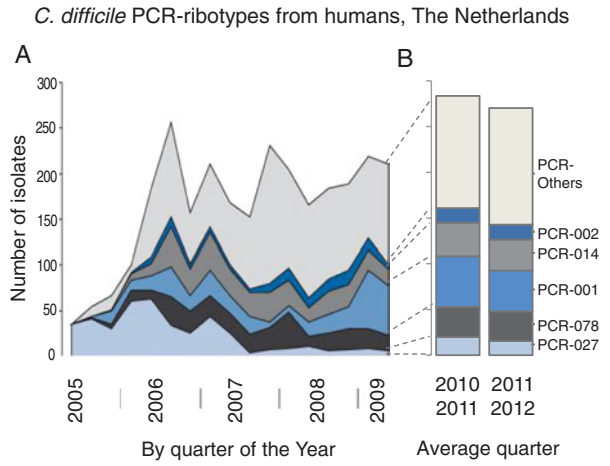

Fig. 3. The same strains that have been observed in animals and foods (see fig. 4) have been predominant and responsible for a major fraction of severe CDIs in people. Note seasonal peaks and that 5 (of over 300 possible) UK PCR ribotypes have accounted for over half of all human cases despite hospital infection control efforts (see Seasonality section below). A) Reproduced with permission from Hensgens *et al.,* 2009, Copyright Eurosurveillance. B) Note increase of PCR-027 (Courtesy Dr. EJ Kuijper, personal communication). Compiled from Hensgens *et al.,* 2011, 2012).

about 7% (Rodriguez *et al.*, 2012). Younger cattle used for food production, i.e. veal calves, although representing <2% of all meat consumed in the USA, can also have *C. difficile* strains of relevance for disease in humans (Costa *et al.*, 2011; Houser *et al.*, 2012). Regardless of its association with enteric disease, *C. difficile* isolates derived from cattle were the first to draw attention to the potential for foodborne transmissibility involving current epidemic human strains PCR ribotypes 017, 027, 077, 014, and 078 (Rodriguez-Palacios *et al.*, 2006, 2009, 2012; Keel *et al.*, 2007; Hammitt *et al.*, 2008) (Compare Fig. 3 and 4). Antimicrobial resistance against new-class linezolid, but not tigecycline, has been observed in *C. difficile* from cattle at harvest in the USA (Rodriguez-Palacios *et al.*, 2011a).

Food animals – poultry

This is the food animal species that has been studied the least. The first studies that highlighted the potential relevance of poultry as carriers of toxigenic strains are from Africa (Simango, 2006; Simango and Mwakurudza, 2008). In Zimbawe, Simango and colleagues showed that up to 30% of free-range chickens carried toxigenic *C. difficile* with antimicrobial resistance patterns of relevance for humans (Simango, 2006; Simango and Mwakurudza, 2008). These results indicate that the risk of transmission via foods/animals to susceptible people in Africa, where the rate of HIV-infected patients in the community at risk for CDI is high (Onwueme *et al.*, 2011), might be a relevant factor to consider for targeted intervention. The prevalence of *C. difficile* in free range poultry in Zimbabwe could be extrapolated to comparable societies where free range poultry is common practice including some Asian and Latin American countries where *C. difficile* has been problematic in humans (Legaria *et al.*, 2003; Rupnik *et al.*, 2003; Huang *et al.*, 2008; Balassiano *et al.*, 2012).

Recent studies on poultry commercial operations have also documented the relevance of *C. difficile* in Europe and North America, where free-range production contributes less to the food supply. In Slovenia, one study conducted on an intensive commercial farming system reported that the percentage of birds colonized with *C. difficile* was higher than that reported in African free-range poultry, with prevalence decreasing with age (Zidaric *et al.*, 2008), as shown in calves, other animals, and children (Rodriguez-Palacios *et al.*, 2006; Enoch *et al.*, 2011). In that study, over 60% of birds carried *C. difficile* in early production, but it was significantly less frequent (below 2%) as animals approached harvest time. In Austria, 5% of poultry tested had *C. difficile* (Indra *et al.*, 2009). In agreement, the percentage of poultry and turkey colonized at harvest in the USA approached zero in intensive rearing facilities in Ohio (Rodriguez-Palacios *et al.*, unpublished data). More recently, the prevalence of *C. difficile* in commercial chickens was also comparable at 2% prior to harvest in Texas in a study conducted by the U.S. Department of Agriculture (Harvey *et al.*, 2011). Clearly, much more work needs to be done in this field also, especially because, inexplicably, the prevalence of *C. difficile* in poultry meats, at least in North America, is significantly higher postharvest, ranging between 6 and 12% of chickens (Weese *et al.*, 2010b; Harvey *et al.*, 2011). Considering that *Campylobacter* illnesses in humans are often associated with the consumption of poultry (Moran *et al.*, 2009; Scallan *et al.*, 2011), it is possible that the risk of infection with *C. difficile* via ingestion of contaminated foods is comparable, simply because spores are expected to be more resistant to heat than the vegetative and viable but not culturable forms of *Campylobacter* spp.

In summary, from the available reports of *C. difficile* in animals, two general conclusions can be drawn. First, similar to humans, newborn and young animals are more frequently colonized by *C. difficile* than adult animals; however, unlike humans neonatal animals are at higher risk of being affected with enteric disease. Second, also similar to humans, animals in most studies exhibit a small diversity of *C. difficile* strains, although the high strain diversity observed in some cattle and poultry studies (Rodriguez-Palacios *et al.*, 2006; Zidaric *et al.*, 2008; Avbersek *et al.*, 2009) could be a reflection of culture methodology and farm variability. High strain diversity usually indicates successful colonization with minimal selection forces. Environmental and host associated

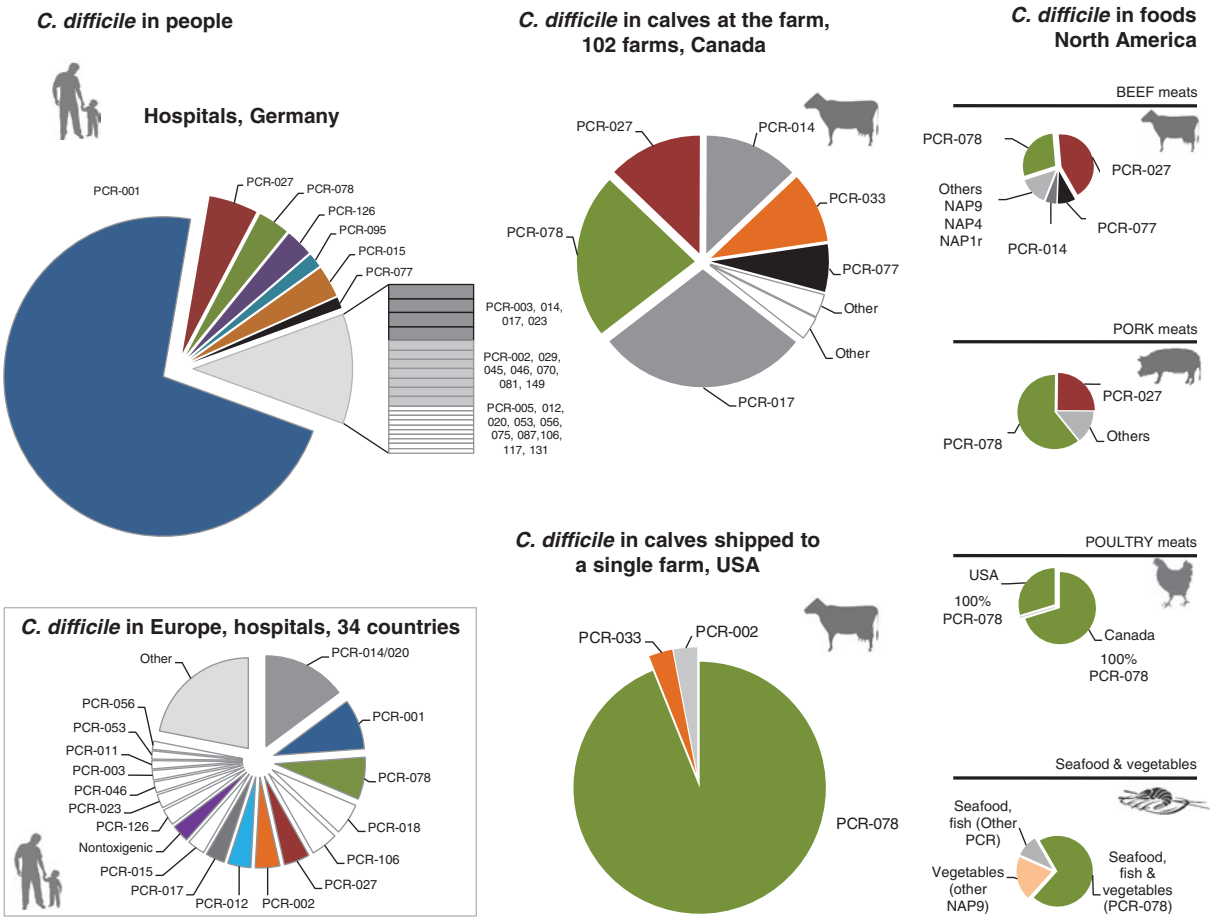

Fig. 4. Frequency of isolation of toxigenic *C. difficile* of distinct PCR ribotypes from humans, young cattle, and various foods. Note the relative importance of food animal derived strains in cases of human disease, and the presence of some ribotypes in Europe and North America (i.e., PCR-078). Data compiled from Rodriguez-Palacios *et al.*, 2006; Keel *et al.*, 2007; Bauer *et al.*, 2011; Reil *et al.*, 2011; Hensgens *et al.*, 2012.

factors are possibly contributing to the selection of a few predominant strains.

Waters and the environment

In general, spore-forming bacteria including clostridia are microorganisms that last a long time in the environment. With very few exceptions, *C. difficile* produce spores that can survive for months in the environment (Baverud *et al.*, 2003), but not many publications are available in this regard. A British publication described the presence of toxigenic *C. difficile* in soils, wells, recreational waters, veterinary clinics, and in households (al Saif and Brazier, 1996). In Africa, soils in rural areas of Zimbawe inhabited by free-range chicken also had toxigenic *C. difficile* (Simango, 2006). Despite these publications, little attention has been placed on the environment as a source of infectious spores and on its role in human and animal infections. *C. difficile* spores are disseminated via air in indoor environments (Roberts *et al.*, 2008) (see Dissemination below). At the farm level, this area of research remains largely unexplored.

C. difficile in foods

Discovering that a particular microorganism becomes an emerging food safety concern should not be surprising. Foods have been a historic source of exposure for many pathogens. However, despite the expectedness of such an event, skepticism is natural. *C. difficile* was first recovered from foods and animals in 1980. In 1982, it was suspected as the cause of PMC that occurred after an elderly patient consumed canned salmon (Gurian *et al.*, 1982). However, the patient had other health issues (hypochloremia) and the food item was not cultured. During 1981–1983, two studies reported finding no *C. difficile* in cooked foods from hospital menus; but the studies did not acknowledge that fresh foods or undercooked foods could be a source of exposure. It is important to note that culture methodology for food and environmental samples might have been suboptimal at the time since concurrent sampling of hospital air and walls yielded no *C. difficile* in the same studies. Further discussion about potential foodborne transmission went on until 1983 (Borriello *et al.*, 1983a).

In retrospect, we now know that thorough cooking (at least 96°C, 15 min) should eliminate the amount of *C. difficile* expected to be found in most foods (Rodriguez-Palacios and LeJeune, 2011). During the 20-year period 1982–2002, there was only one publication on *C. difficile* and foods. It was a report of an incidental finding of *C. difficile* in packed meats published by Broda *et al.* (1996).

Raw and ready-to-eat foods

Raw ground beef and pork were among the first food products to be found contaminated with *C. difficile*. In 1994, Broda and her colleagues, studying microorganisms that caused gas 'blown pack' spoilage in ready-to-eat meats incidentally found *C. difficile* (Broda *et al.*, 1996). The next study conducted on raw meat commercial diets for dogs and cats also found *C. difficile* in a sample of turkey-based diet (Weese *et al.*, 2005). Despite the frequent occurrence of *C. difficile* in foods, its public health significance has generally been under-recognized or viewed with skepticism. A specially designed study base on MLVA have highlighted that the isolation (and prevalence) of *C. difficile* in the food supply is real and not due to laboratory contamination of the food samples (Curry *et al.*, 2012).

In 2007, the first study documenting human epidemic strains of *C. difficile* in foods (specifically, in 20% of retail ground meats), documented the regional and international relevance of the finding (Rodriguez-Palacios *et al.*, 2007a). Subsequent studies have confirmed that this pathogen can be found in other foods tested. Now, scientific reports describe toxigenic *C. difficile* in meats in several countries. Although the percentages of meat packages that have been contaminated with *C. difficile* have ranged from 3 to 42%, the overall expected real prevalence of *C. difficile* contamination under natural conditions at the store level (by sampling 1–2 retail packages of meat per store) has been determined to be at about 6% (Rodriguez-Palacios *et al.*, 2009). Poultry has been the type of meat least studied. One study found no *C. difficile* in retail poultry (Indra *et al.*, 2009); however recent studies indicate that poultry meats can also carry toxigenic strains (Weese *et al.*, 2010b; Harvey *et al.*, 2011). In the USA, the frequency of contamination of retail chicken has been documented to be between 9 and 18%, with all the edible animal parts (legs, wings, thighs, etc.) having comparable frequencies of contamination (Weese *et al.*, 2010b). Of concern, emerging *C. difficile* PCR ribotype 078 strains was found in retail chicken in both Canada and the USA (Weese *et al.*, 2010b; Harvey *et al.*, 2011). This strain is an emerging strain in humans, in hospitals, and the community, in food production environments and in retail foods (Rupnik *et al.*, 2008). The earlier identification of *C. difficile* in animals, with the subsequent increase of incidence of PCR ribotype 078 among people with CDI over the last decade indicates that this pathogen strain is likely moving from animals to humans (Goorhuis *et al.*, 2008; Hensgens *et al.*, 2012). At the processing plant, there is now molecular evidence to suspect persistence and potential cross-contamination of retail food (pork) products with unique MLVA types belonging to the PCR ribotype 078 clone over time (Curry *et al.*, 2012).

Convenient ready-to-eat products (deli meats and minimally processed fruits and vegetables) are gaining market share. Unlike other infamous foodborne bacteria, such as *Escherichia coli* O157:H7, the spores formed by *C. difficile* that are often found in these products are highly resistant to current recommended cooking food safety guidelines. Molecular studies confirmed in Scotland that ready-to-eat salads were contaminated with *C. difficile* strains linked to human disease (Bakri *et al.*, 2009). *Clostridium difficile* was first isolated from root vegetables in 1996 (al Saif and Brazier, 1996). More recently, it has been isolated from vegetables in North America; (J. G. Songer, 2007, personal communication; Rodriguez-Palacios and LeJeune (2007), unpublished data; Metcalf *et al.*, 2010). *C. difficile* have also been isolated from shellfish and fish, which are often consumed undercooked or raw (Metcalf *et al.*, 2011). In Europe, the highest rate of food contamination was reported last year in edible mollusks in Italy, 49% (Pasquale *et al.*, 2012). Several reviews are available summarizing the studies documenting *C. difficile* in foods (Indra *et al.*, 2009; Gould and Limbago, 2010; Weese, 2010). In Latin America, the first report is from Costa Rica, where a molecular clinical genotype was found in 2% of food samples; notoriously, the isolates were susceptible to the antibiotics to which the clinical isolates were highly resistant (Quesada-Gomez *et al.*, 2013). Unless proven otherwise, antimicrobial discrepancy between genetically related strains should not be used to deem two isolates as non-related (Eyre *et al.*, 2012).

Seasonality

As with many other diseases, there could be parallel in the seasonal trends in CDI associated with the prevalence of the causative bacterium in animals, foods and humans (Rodriguez-Palacios *et al.*, 2009). The number of cases of CDI in humans is higher during winter months, at least in northern latitudes (Burckhardt *et al.*, 2008; Rodriguez-Palacios *et al.*, 2009; Reil *et al.*, 2012). That seasonal increase has been partly attributed to a larger number of cases associated with seasonal respiratory and enteric viral infections that require antimicrobial administration or hospitalization (Polgreen *et al.*, 2010). In foods and food animals, at least three independent studies document the same seasonal pattern in North America (higher prevalence in winter) (Rodriguez-Palacios *et al.*, 2006, 2009; Norman *et al.*, 2009; Kho 2012). Although seasonality patterns could occur independently in parallel as a function of climatic variations (Naumova *et al.*, 2007), it is

also possible that the prevalence of *C. difficile* at least in food animals, some foods, and people could be epidemiologically connected. It is important to note that earlier studies did not identify seasonal patterns in human disease (Tvede *et al.*, 1990).

Together, the molecular characteristics and virulence markers of food and food animal-derived *C. difficile* isolates indicate that the presence of emerging strains in vegetables and meats (and possibly the seasonality) might have a direct, yet unproven, connection with the epidemiology of CDI in humans. Although confirming such a connection might take some time, there is enough epidemiological evidence to take action and enhance prevention through education to minimize the risk of inadvertent exposure to *C. difficile* among individuals at risk.

Irrespective of the type of food product tested, the most important and concerning finding is that emerging hyper-virulent strains of *C. difficile* (PCR ribotypes 027 and 078) are among the most predominant genotypes recovered from foods (Fig. 4). The reasons for the predominance of these ribotypes are unknown, but increased sporulation rates could favor some strains (Akerlund *et al.*, 2008) to become endemic in the environment.

Dissemination of C. difficile

More recently there have been growing concerns regarding biosecurity and further global dissemination (Clements *et al.*, 2010). Hyper-virulent strains of *C. difficile* that were first reported in humans and animals in Eastern North America and Western Europe in the early 2000s (Warny *et al.*, 2005; Kuipjer *et al.*, 2008) have been identified in sporadic cases and outbreaks of disease in humans in more distant locations, including Australia, Japan, Korea, and Singapore, since 2007 (Sawabe *et al.*, 2007; Tae *et al.*, 2009; Clements *et al.*, 2010; Lim *et al.*, 2011). Transcontinental commercial flights and the importation of live animals from places where emerging strains are documented have been listed as possibilities for dissemination (Clements *et al.*, 2010) of lineages that emerged in North America (He *et al.*, 2012). At the regional level, studies in white-tailed deer (common visitors to livestock grazing areas and abundant in North America, Europe, and New Zealand) and wild birds have been documented to be an important factor for *C. difficile* dissemination in a suburban agricultural region, with tangible exposure potential to humans and animals in the USA (Rodriguez-Palacios *et al.*, unpublished data; French *et al.*, 2010). *C. difficile* has been isolated from several other wildlife species since the 1980s, including feral swine populations (Thakur *et al.*, 2011). Air dissemination studies have increased in recent years. Studies conducted around the vicinity of pig farms indicate that aerial dissemination for short distances is possible with downstream currents. In bathrooms, *C. difficile* has been found surrounding toilets, presumably due to aerosolization of fecal particles during flushing. Not surprisingly, this is of preventive relevance since seemingly identical *C. difficile* strains have been isolated from pigs and from toilets used by the farm workers in an integrated swine operation (Norman *et al.*, 2009).

Genome association studies

Whole-genome, microarray-based studies indicate that food animals might have been the original sources of some emerging epidemic strains of *C. difficile*, particularly newly emerging PCR ribotype 078 (Stabler *et al.*, 2006; Goorhuis *et al.*, 2008; Bakker *et al.*, 2010). MLVA analysis continues to indicate that hospitalized humans and food animals, and foods are carrying clonally related strains (Marsh *et al.*, 2011; Koene *et al.*, 2012). However, no conclusive studies are available to determine if animal shedding or food contamination are associated with changing patterns of disease in humans. Rather, it is possible that the pathogen constantly moves between humans, animals, and the environment, partly evolving and adapting as it moves across temporal and spatial niches. Given the spore forming nature of *C. difficile*, it is possible that inter-species transmission occurs from environmental sources and that some level of host adaptation (Janvilisri *et al.*, 2009) and clonality has also ensued in parallel over millions of years (Stabler *et al.*, 2006; He *et al.*, 2010). Horizontal gene transfer and homologous recombination are very frequent genetic events in *C. difficile*. It is possible that the epidemiology of *C. difficile* will continue to evolve. Genomic approaches are increasingly used to understand virulence pathways and to provide modern alternatives for rapid diagnosis and treatment (Forgetta *et al.*, 2011; Eyre *et al.*, 2012), but prevention strategies remain a challenge mostly due to limited information on disease ecology (outside hospitals) and inherent problems with integration of knowledge across disciplines. Addressing this issue, here we identify areas where recommendations should be expanded (Table 3). We have also proposed a list of simple educational measures for prophylactic use, which is under multidisciplinary consideration.

Reducing risks by targeted prevention in the community

Many aspects of ecology and epidemiology of *C. difficile* are still unknown. Achieving an increased understanding of the factors that contribute to the survival and persistence of this organism in different environments is a critical step to enhance environmental health, food safety, and disease prevention. Reducing the presence of this pathogen at preharvest, harvest, and postharvest stages of food production will allow the

Table 3. Need of new and improved recommendations to reduce exposure to *C. difficile*

Currently publicized:

- Hand washing – Requires emphasis for people at risk and for food/animal health professionals.
- Use of antimicrobials and antacids – Requires emphasis to target susceptible communities outside hospitals, and involvement of pharmacists.

Not existent, not publicized:

- Contact precautions regarding human and animals with CDI, healthy pets and wild animals.
- Cleaning and disinfection – Addressing food, home, kitchen and laundry environments.
- Thorough cooking – Current food safety guidelines are ineffective against *C. difficile*.

development of science-based strategies to prevent food contamination. Meanwhile, if foodborne transmission of this important pathogen is significant, cooking, and hygiene measures to enhance the elimination or destruction of *C. difficile* spores from potentially contaminated retails foods or from areas where food is prepared could mitigate the incidence of human disease. Although no infective dose data are available for humans, the number of spores needed for infection is presumed to be small based on CDI hospital epidemiology and studies with animals. Considering that (1) immune-compromised laboratory mice require about two environmental *C. difficile* spores/cm^2 to become ill (Lawley *et al.*, 2009), (2) that contaminated foods carry 20 to 240 *C. difficile* spores per gram (Weese *et al.*, 2009, 2010b), and (3) that infected healthy animals shed between 1,000 and 10,000 spores per gram of feces (Rodriguez-Palacios *et al.*, 2011), it is important to emphasize the need of new and expanded measures to reduce pathogen exposure (Table 3), which is necessary for CDI induction. Not only would these measures impact the exposure to *C. difficile* complementing existing infection control guidelines (Gerding *et al.*, 2008), enhanced food hygiene and thorough cooking would also reduce illnesses associated with other enteric pathogens.

Recommending thorough cooking, kitchen hygiene, and minimize exposure

To date, most food safety guidelines available to the community instruct people to cook most foods at determined minimum internal temperatures to achieve a significant (6 log units) reduction of major foodborne pathogens to make most meals safe. These ranges vary from 63°C to 74 or 85°C (CFIA, 2010; USDA, 2011). Because recent quantitative studies have shown that *C. difficile* spores can survive extended heating at 71°C (160°F), the minimum temperature recommended for cooking of meats (Rodriguez-Palacios *et al.*, 2010), it is necessary to heat foods at higher temperatures to inactivate *C. difficile* spores. Based on quantitative analysis with *C. difficile* isolates derived from foods, food animals, and humans (Meisel-Mikolajczyk *et al.*, 1995; Rodriguez-Palacios and LeJeune, 2011), heating foods to 85°C for 10–15 min could be a reasonable strategy to minimize the counts of *C. difficile* in foods. Alternatively, heating at 96°C (sub-boiling) could reduce 6 $\log_{10}$ within 2–3 min (Rodriguez-Palacios and LeJeune, 2011). Thorough cooking at boiling temperatures, a common household practice, is ideal, and could be emphasized. As *C. difficile* could still survive cooking temperatures and multiply in heated foods, it is also recommended that foods be properly chilled and stored as indicated for other clostridial foodborne pathogens.

Conclusion

C. difficile has been associated with disease in people since 1975, but recently the identification of emerging multidrug resistant hyper-virulent strains from animals and foods indicate that there is the potential risk for transmission and infection in humans, especially among high-risk populations. Since CDIs have been traditionally considered as hospital acquired diseases, little attention has been paid to the sources of infection and risk factors in the community. Community-onset CDIs as they are admitted to health care centers have the potential to influence the overall epidemiology of this disease. Although there are no scientific reports explicitly confirming that *C. difficile* can be acquired via foods or contact with animals, there is sufficient laboratory and epidemiological research data and the mechanistic rationale (i.e. principles of fecal–oral transmission of enteric pathogens) to propose and adopt interventions to prevent transmission. Understanding the risk factors associated with disease and the sources of *C. difficile* where the pathogen is acquired by food animals and by humans can assist in developing strategies to enhance food safety and protect human health. Prevention at various levels is especially important as the theory of person-to-person transmission is being reexamined.

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