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Clostridium difficile in food—innocent bystander or serious threat?

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Abstract

Clostridium difficile is a critically important cause of disease in humans, particularly in hospitalized individuals. Three major factors have raised concern about the potential for this pathogen to be a cause of foodborne disease: the increasing recognition of community-associated *C. difficile* infection, recent studies identifying *C. difficile* in food animals and food, and similarities in *C. difficile* isolates from animals, food and humans. It is clear that *C. difficile* can be commonly found in food animals and food in many regions, and that strains important in human infections, such as ribotype 027/NAP1/toxinotype III and ribotype 078/toxinotype V, are often present. However, it is currently unclear whether ingestion of contaminated food can result in colonization or infection. Many questions remain unanswered regarding the role of *C. difficile* in community-associated diarrhoea: its source when it is a food contaminant, the infective dose, and the association between ingestion of contaminated food and disease. The significant role of this pathogen in human disease and its potential emergence as an important community-associated pathogen indicate that careful evaluation of different sources of exposure, including food, is required, but determination of the potential role of food in *C. difficile* infection may be difficult.

Keywords: *Clostridium*, foodborne, review, zoonotic

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Introduction

Clostridium difficile is a Gram-positive, anaerobic, spore-forming bacterium that has come to the forefront as an important human pathogen. It was initially dismissed as a commensal in healthy infants [1], but was recognized as an important cause of antimicrobial-associated diarrhoea in the 1970s. It is now the most commonly diagnosed cause of antimicrobial-associated and hospital-associated diarrhoea, and the cause of virtually all cases of pseudomembranous colitis [2].

The normal location for *C. difficile* is the intestinal tract of humans and various animal species. The pathophysiology of *C. difficile* infection (CDI, formerly referred to as *C. difficile*-associated diarrhoea) is only partially understood, and involves overgrowth of toxigenic strains of *C. difficile*, followed by production of toxins and the development of a range of clinical signs, from mild self-limiting diarrhoea to life-threatening pseudomembranous colitis, toxic megacolon and/or intestinal perforation [2,3]. It is assumed that disruption of the normal protective gastrointestinal microflora is an important factor for *C. difficile* overgrowth and disease [4]. Antimicrobial therapy is the most widely reported risk factor [5,6], but other risk factors have been identified [7,8], and an inciting cause is not apparent in all cases.

The main virulence factors that are currently recognized are two large clostridial toxins, toxin A (TcdA, an enterotoxin) and toxin B (TcdB, a cytotoxin) [9]. A third, large, unrelated toxin, designated *C. difficile* binary toxin (CDT), can also be produced by some strains [9–11]. The role of binary toxin in disease is currently unclear [12,13], but there is information suggesting that this toxin may be clinically relevant [14]. Most toxigenic strains produce both TcdA and TcdB. A small percentage of clinically relevant strains produce TcdB but not TcdA [15]. Previously, the prevalence of

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binary toxin-producing strains was low (<10%) [10,11]; however, this has increased in recent years, and binary toxin-producing strains now represent more than 30% of isolates from humans in some studies [13,16,17].

Typing and Nomenclature

C. difficile typing techniques and nomenclature are not standardized; various systems are used, which can result in difficulties in comparing different studies. PCR ribotyping, pulsed-field gel electrophoresis (PFGE) and toxinotyping are currently the main typing techniques. Accordingly, strains are often classified using a combination of names from different typing methods. Two types currently receive the greatest attention. One is termed ribotype 027 or NAP1 (North American pulsovar 1) according to PFGE. It is a toxinotype III strain that has genes encoding TcdA, TcdB and CDT. It also has an 18-bp deletion and upstream mutation in *tcdC*, a toxin regulatory gene, which has been associated with increased production of TcdA and TcdB *in vitro* [18]. This strain has been implicated in outbreaks of severe disease internationally [13,19,20], and is also a common endemic strain in many regions [16,21,22]. Another potentially important strain, particularly in community-associated disease, is ribotype 078, a toxinotype V strain that corresponds with NAP7 or NAP8 [23]. Like ribotype 027, this strain possesses genes encoding TcdA, TcdB and CDT. It also has a deletion in *tcdC*, but of 39 bp, and a mutation in a different upstream region. This strain produces more TcdA and TcdB *in vitro* than strains with wild-type *tcdC*, but less than ribotype 027 [24]. However, it is unclear whether this toxin overproduction contributes to increased virulence *in vivo*.

Changing Epidemiology in Humans

There have been two main changes in the epidemiology of CDI over the past 10 years. The first was an increase in the incidence and severity of hospital-associated CDI, with large outbreaks, high mortality rates, and poorer response to treatment, reported internationally [13,19,20,25]. This has been largely attributed to the emergence and dissemination of ribotype 027/NAP1 but other factors, such as the increasing use of fluoroquinolones, may also be involved [5,26].

The second apparent change has been the increasing recognition of community-associated (CA) disease. This includes disease in young individuals and other people traditionally considered to be at low risk [27,28], although there is some debate as to whether this is an emerging problem or whether it was overlooked in the past. Along with increasing recognition of CA CDI, an increase in the prevalence of toxinotype V strains has been observed, particularly ribotype 078: from 3% to 13% in The Netherlands [29], from 3.3% to 11% in France [30], and from <0.02% to 1.3% in the USA [24]. There are also indications that these strains may be over-represented in CA CDI [24,29].

C. difficile in Food Animals

Concerns regarding the potential for foodborne transmission of *C. difficile* inevitably arose following isolation of *C. difficile* from the faeces of food animals (cattle, pigs and chickens) in various countries (Table 1).

The role of *C. difficile* in animal disease varies with species, with a clear role of enteric disease in young piglets [31,32], and less convincing evidence for calves [33–35]. The major public health concern, however, involves shedding of *C. difficile* by clinically normal animals, as that population could constitute a large and inapparent reservoir of this pathogen for contamination of food, water and the environment.

Although data regarding *C. difficile* in food animals are compelling, care must be taken when interpreting currently available studies. Only a limited number of studies have been published, and these have typically involved a small number of geographical regions, with different sampling methods and culture techniques. These methodological variations preclude

TABLE 1. Prevalence of isolation and ribotype distribution of *Clostridium difficile* from food animals and retail meat

Country	Sample type	Prevalence (%)	Ribotype 027/ toxinotype III (%)	Ribotype 078/ toxinotype V (%)
Canada [34]	Calves	15	12	26
USA [33]	Calves	25	0	94
Canada [40]	Veal calves	49	0/1	65
Slovenia [61]	Calves	1.8	0	0
Austria [49]	Cows	4.5	0	0
Slovenia [36]	Chickens	62	0	0
Austria [49]	Chickens	5	0	0
Zimbabwe [62]	Chickens	29	NT	NT
Slovenia [61]	Piglets	52	0	0/77
USA [32]	Piglets	79	NT	NT
USA [63]	Piglets	NA	0	83
Austria [49]	Pigs	3.3	0	0/50
Canada [37]	Piglets	95	0	94
Canada [44]	Beef, veal	20	0/67	0
USA [23]	Various	42	27	73
Canada [45]	Beef, veal	6.1	0/27	0
Canada [46]	Pork	1.8	43/57	0
Canada [47]	Chicken	15	0	96
Canada [48]	Pork	12	7.1/14	71
	Beef	12	7.1	86

NT, typing was not performed; NA, not applicable, as the study was an evaluation of previously collected isolates.

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the comparison of prevalence data from different studies. Furthermore, it is apparent that there is significant variation in *C. difficile* colonization among different age groups in calves, piglets and chickens, with *C. difficile* rates decreasing substantially over time [36, 38–40].

For example, a longitudinal study of pigs in one swine operation noted 50% colonization of suckling piglets, but only 8.4% in weaned pigs and 3.9% in grower-finisher pigs [38]. This age effect needs to be considered, as the main risk in terms of foodborne disease is shedding from animals around the time of slaughter, not when they are neonates, so specific details about sample population must be considered when evaluating different studies. Because of the apparent effect of age on prevalence, studies of animals close to the time of slaughter would be most appropriate for assessment of foodborne risks, as opposed to studies of other age groups. Nevertheless, despite these limitations, it is clear that *C. difficile* can be found in food animals in some countries, and it is therefore reasonable to assume that similar results would be found in many, if not all, other countries.

Characterization of *C. difficile* strains found in food animals has given further support to concerns about the potential for foodborne transmission, particularly the common finding of ribotype 078/toxinotype V and isolation of ribotype 027/NAP1/toxinotype III or related strains (Table 1). Although the limitations discussed above regarding assessment of prevalence should also apply to distribution of strains, some trends are apparent, particularly the high frequency of isolation of ribotype 078/toxinotype V from different food animals. However, it should be noted that, despite frequent referral to ribotype 078/toxinotype V as a 'food animal strain', it is not the only strain found in food animals, and nor is it the most common in all studies. Current data strongly suggest that this strain is over-represented in food animals, but, again, the small number of studies and variability in results indicate that caution should be exercised when making broad statements about strain origin. Reports of ribotype 027 in food animals have raised significant concerns because of the importance of this strain in human disease, including large outbreaks.

The original source of *C. difficile* in food animals is unknown. Finding the same strains of *C. difficile* in food animals and humans strongly suggests that either one was the source of infection of the other, or that some other common source resulted in infection of both populations, as convergent evolution of the same strains in people and food animals would probably be less likely. A study of human and food animal isolates of ribotype 078 using highly discriminatory methods such as multilocus variable-number tandem repeat analysis has revealed indistinguishable isolates from pigs and humans [41], providing further support for the likelihood of interspecies transmission, but no inference as to the original source.

C. difficile in Retail Meat

The first report of *C. difficile* in retail meat was a rather vague report, describing the isolation of *C. difficile* from spoiled vacuum-packed meat samples [42], which received little attention. A study finding *C. difficile* in raw meat diets intended for dogs [43] received similarly little attention, despite the fact that, in hindsight, these two studies indicated that it was likely that *C. difficile* would be found in retail meat. The first specific investigation of *C. difficile* contamination of retail meat intended for human consumption was a study from Canada in 2007 [44]. That study involved a convenience sample of ground beef (n = 53) and veal (n = 7) from five stores in two Canadian provinces. By enrichment culture, *C. difficile* was isolated from 12 of 60 (20%) samples (21% ground beef and 14% ground veal). The most common strain, accounting for 67% of isolates, was a toxigenic strain that possessed genes encoding TcdA, TcdB and CDT, belonged to toxinotype III, and had an 18-bp deletion in the *tcdC* gene. This strain was classified as NAP1 by PFGE but, although it was similar to ribotype 027 in all other respects, it had a different ribotype pattern. The other two toxigenic ribotypes were types previously identified in humans in Canada.

A similar study from the USA, using convenience sampling from stores in the Tuscon, Arizona area, reported isolation of *C. difficile* from 37 of 88 (42%) samples, including ground beef (13/26, 50%), summer sausage (1/7, 14%), ground pork (3/7, 43%), braunschweiger (10/16, 63%), chorizo (3/10, 30%), pork sausage (3/13, 23%) and ground turkey (4/9, 44%) [23]. Ribotype 078 was the most common strain, accounting for 73% of isolates, with the remaining isolates belonging to ribotype 027. A subsequent Canadian study was performed with a systematic sampling method to obtain retail meat samples from three provinces over an 8-month period [45]. The use of systematic national sampling instead of geographically focal convenience sampling is likely to provide a better estimate of true population prevalence. *C. difficile* was isolated from ten of 149 (6.7%) of ground beef samples and three of 65 (4.6%) of veal chop samples, giving a combined prevalence of 6.1%. This study used three different culture techniques, and the prevalence of recovery obtained using the different methods ranged from 1.4% to 2.3%, with poor agreement among methods. No methods were identified as being superior to others, and it was hypothesized that the inter-assay variability could have been related to low levels

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of non-homogenously distributed spores in samples. Isolation of *C. difficile* was more common in February than in the other months, although the reason for this apparent seasonality was unclear. Seventy-seven per cent of isolates were toxigenic, and all of these had been previously recovered from humans in Ontario [16]. Two NAP1/toxinotype III strains that were different from ribotype 027 accounted for 31% of toxigenic isolates, ribotype 077/NAP2 accounted for 23%, and ribotype 014/NAP4 accounted for 15%. A later study of pork in Canada, using systematic sampling, reported isolation of *C. difficile* from only 1.8% of ground pork and pork chops [46].

The most common strain was ribotype 027/NAP1, which accounted for 43% of isolates. Interestingly, ribotype 078 was not identified in any of the first three Canadian studies. Fifteen per cent (25/162) of the chicken meat samples yielded *C. difficile* in another Canadian study involving sampling from four provinces [47].

As opposed to what was found earlier studies, 96% of isolates were ribotype 078, and the remaining isolate was a toxinotype 0/NAP4 strain that had been previously found in humans. In contrast to earlier studies, a more recent Canadian study identified ribotype 078 as the predominant strain in beef (86%) and pork (71%) [48].

The reasons for the apparent changes in strain distribution among the Canadian studies are unclear. Considering that sampling for most studies involved a national surveillance programme, sampling bias is less likely, and it is possible that these changes could have reflected a dynamic event of ribotype 078 emergence in food animals in Canada; however, this cannot be proven.

In contrast to the above studies, *C. difficile* was not isolated from any of 51 beef, 27 pork and six chicken samples in Austria [49]. As with studies of food animals, care should be taken in comparing different studies of retail meat. The use of different sampling and culture methods precludes objective comparison of prevalence. However, it remains noteworthy that *C. difficile* can be found relatively commonly in various meat products from different regions, and that strains found in retail meat are usually strains that are also found in humans. Standardization of sampling, culturing and identification methods would therefore be highly welcome.

A limitation of the initial studies was their reliance on enrichment methods. While appearing logical, based on the desire to have optimal recovery in order to estimate the prevalence of contamination, enrichment culture results in incomplete information, particularly with respect to the amount of *C. difficile* that is present. The infective dose of *C. difficile* for humans is not known, and it probably varies among individuals, but an understanding of the degree of contamination is critical for assessment of risk. Quantification of contamination has only been reported in one study. That study evaluated beef and pork from four Canadian provinces, using enrichment and quantitative methods [48].

The enrichment method was shown to have a sensitivity of ≤10 spores/g, a confirmation that enrichment methods can indeed reveal a very low level of contamination. Whereas *C. difficile* was isolated from 12% of both beef and pork samples, 71% of the positive samples were positive only when enrichment culture was used. Of the samples for which quantification was possible, contamination ranged from 20 to 60 spores/g in pork and from 20 to 240 spores/g in beef. This suggests that, although contamination may be relatively common, spore numbers tend to be low. Interestingly, that study also found a small percentage of samples that were positive using quantitative culture but not enrichment culture, which could indicate non-homogeneous distribution of spores in meat.

On the basis of these preliminary studies, there appears to be a discrepancy between the strain distribution of *C. difficile* in meat and that in food animals (Table 1). Specifically, the prevalence of ribotype 027/NAP1 appears to be disproportionate in food as compared with food animals. It is possible that this is simply a function of the small number and scope of current studies, and that broader studies will reveal a clearer link between food animals and food strains. However, it is also possible that the strain distribution in faeces of food animals is not the only factor determining the strain distribution in food. Although the gastrointestinal tract is presumably an important source of *C. difficile* contamination, other sources must also be considered. These include the animals' hides, the slaughterhouse environment, the processing facility environment, processing equipment, the hands of personnel manipulating meat, and any other environment where meat is handled or processed prior to sale (e.g. butcher's counter in a grocery store). *C. difficile* spores are highly resistant to most disinfectants, and therefore could survive common cleaning and disinfection practices, and persist or accumulate in the environment. Ribotype 027/NAP1 may be particularly adept at persisting in the hospital environment, because of its high sporulation rate [50]. It is plausible, although completely hypothetical at this point, that the high sporulation rate of ribotype 027 could also be an important component of environmental persistence in slaughterhouses and meat-processing environments. Better environmental persistence could result in over-representation of this strain in meat. Therefore, studies of *C. difficile* contamination of slaughterhouse and processing facilities are required, as are longitudinal studies of the entire process from 'farm to fork', to determine all stages at which

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contamination can occur, and their relative importance. Importantly, this should lead to identification of critical points where interventions could be applied, if necessary.

Another potential source of infection that requires investigation is the presence of *C. difficile* spores in healthy muscle tissue in living animals. A study of healthy equine muscle found clostridial spores in 19% of samples; however, none were *C. difficile* [51]. A pilot study of bovine muscle found similar rates of clostridial spores in healthy muscle, including isolation of *C. difficile* spores from one cow (J. S. Weese, unpublished data). The origin of spores in healthy muscle has not been investigated, but it is possible that transient bacteraemia, secondary to enteritis at some point in life, may disseminate clostridia to healthy muscle tissue. In this aerobic environment, clostridial spores would remain dormant. Their subsequent contribution to contamination of meat remains unknown but cannot be dismissed, even though levels of *C. difficile* spores in healthy muscle would presumably be very low.

C. difficile in Other Food Products

Although contamination of retail meat has received the most attention, contamination of other food products may be equally important, particularly for those that are eaten after little cleaning or cooking. Minimal investigation of other food products has been reported. A large study of *C. difficile* in South Wales reported isolation of *C. difficile* from seven of 300 (2.3%) vegetables: two potatoes, one onion, one mushroom, one carrot, one radish, and one cucumber [52]. The prevalence of isolation among the different vegetable types was not reported. A recent Scottish study described isolation of *C. difficile* from three of 40 (7.5%) ready-to-eat salads [53].

There is correspondingly little information about the types of *C. difficile* found in other food products. Although 71% of isolates from vegetables from South Wales were toxigenic [52], typing was not reported. All three isolates from the Scottish ready-to-eat salads were toxigenic, with two isolates being classified as ribotype 017 and one as ribotype 001 [53]. Both are important in human disease, with ribotype 001 being the most common ribotype isolated in hospitals in Scotland [54].

There are various possible sources of vegetable contamination, all of which are likely to be ultimately human or animal, such as soil, fertilizer (manure), water, processing environments, and human hands. The relative impacts of these are completely unclear.

Heat Tolerance

Clostridial spores are tolerant of adverse environmental conditions, including heat. Persistence of viable *C. difficile* spores has been demonstrated even after 120 min at 71°C, the recommended internal temperature for cooking ground meat [44]. As cooking foods at recommended temperatures cannot be relied on to kill *C. difficile* (in contrast to enteropathogens such as *Salmonella*, *Campylobacter* and *Escherichia coli*), methods for the prevention of even low-level contamination will be required should *C. difficile* be proven to be a foodborne pathogen.

Evidence for Foodborne CDI

There is currently no objective evidence indicating that *C. difficile* is a foodborne pathogen. However, one must consider that few, if any, studies have made concerted (or any) efforts to investigate this area. Some studies of CA CDI are currently assessing food exposure as a risk factor; however, it may be difficult to determine whether food is indeed a source of infection. One reason for this is the lack of information regarding the pathophysiology of foodborne CDI, should it occur. If, as with most enteropathogens, ingestion of food contaminated with an infectious dose leads shortly thereafter to signs of enteric disease, epidemiological studies of food exposure in CA diarrhoea may be successful in revealing an association. However, if foodborne exposure is only of concern for selected, at-risk populations, an association may be more difficult to identify, particularly if these at-risk populations are not adequately defined and those risk factors queried. Additionally, it is plausible that *C. difficile* ingestion could lead to colonization, with subsequent development of CDI at a later date, particularly if and when specific risk factors are encountered. This potentially indirect or delayed onset of disease could certainly hamper objective determination of foodborne risks; identification of the role of food in disease would be very difficult if the point of exposure may have been days, weeks or months in the past. It is also possible that colonization due to foodborne pathogens could result in transmission to close contacts, either directly or through environmental contamination, and result in subsequent colonization or disease; in this scenario, the food source would not cause disease in the person ingesting it, but could ultimately result in CDI in another individual. This may seem unlikely and would be uncommon, but cannot be dismissed, as it would certainly complicate determination

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of the role of food in CDI. In conclusion, although studies investigating the role of food in CDI are certainly warranted, definitive determination of the role of food in CDI may be very difficult.

Putting it in Context

On the basis of recent studies of retail meat, it appears that exposure to low levels of *C. difficile* is probably a very common event. As CA CDI is not equally common, there is clearly not an obligate association between ingestion of *C. difficile* and development of disease. The complexity of this issue should not be overlooked, and there are probably no simple ways of addressing the question of whether *C. difficile* is a foodborne pathogen. There are major knowledge gaps in our understanding of *C. difficile* and CDI. Current studies, while providing critical preliminary information, have limitations and cannot lead to a secure assessment of the role of *C. difficile* in foodborne disease. Furthermore, consideration of CA CDI must involve more than investigations of meat contamination. Various studies have found that *C. difficile* can be isolated from vegetables [52,53], water [52], the household environment [55], pets [56–58], and healthy humans [59,60]. All of these sources may be as important as meat. It is possible that exposure to *C. difficile* is a regular, if not daily, occurrence, and that food is only one of many possible sources. Studies of CA CDI must therefore take a broad, 'ecological' approach that takes into account all possible sources of exposure.

Future directions

Investigation of the potential foodborne risks concerning CDI is very much in its infancy, and broad areas need to be addressed. Some of the important questions that need to be answered are as follows:

1. What role does *C. difficile* play in CA diarrhoea?
2. Are there food ingestion or contact risk factors for CDI? Do these apply to all individuals or just selected, high-risk individuals?
3. How does *C. difficile* get into food? Is the source the animal, slaughterhouse environment, processing environments, hands of personnel at any level, or all of the above?
4. What is the dose of *C. difficile* required to cause disease in healthy and high-risk individuals, and how does that relate to the levels of *C. difficile* found in food?

Conclusion

Is *C. difficile* a serious foodborne threat or an innocent bystander? The answer is currently completely unclear. An evidence-based assessment cannot be performed, given the paucity of data in many areas. There is enough circumstantial evidence to suggest that *C. difficile* could cause a foodborne disease; more careful investigation is required. At the moment, no one can predict with any degree of certainty whether foodborne *C. difficile* will be identified as an important cause of disease, will be completely irrelevant, or will be of concern only to a select group of high-risk individuals.

Transparency Declaration

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References

1. Hall I, O'Toole E. Intestinal microflora in newborn infants with a description of a new pathogenic anaerobe, *Bacillus difficilis*. *Am J Dis Child* 1935; 49: 390–402.
2. Poutanen S, Simor A. *Clostridium difficile*-associated diarrhea in adults. *CMAJ* 2004; 171: 51–58.
3. Borriello S. Pathogenesis of *Clostridium difficile* infection. *J Antimicrob Chemother* 1998; 41 (suppl C): 13–19.
4. Bartlett JG, Perl TM. The new *Clostridium difficile*—what does it mean? *N Engl J Med* 2005; 353: 2503–2505.
5. McCusker M, Harris A, Perencevich E, Roghmann M. Fluoroquinolone use and *Clostridium difficile*-associated diarrhea. *Emerg Infect Dis* 2003; 9: 730–733.
6. Muto C, Pokrywka M, Shutt K *et al.* A large outbreak of *Clostridium difficile*-associated disease with an unexpected proportion of deaths and colectomies at a teaching hospital following increased fluoroquinolone use. *Infect Control Hosp Epidemiol* 2005; 26: 273–280.
7. Dial S, Alrasadi K, Manoukian C, Huang A, Menzies D. Risk of *Clostridium difficile* diarrhea among hospital inpatients prescribed proton pump inhibitors: cohort and case-control studies. *CMAJ* 2004; 171: 33–38.
8. Blot E, Escande MC, Besson D *et al.* Outbreak of *Clostridium difficile*-related diarrhoea in an adult oncology unit: risk factors and microbiological characteristics. *J Hosp Infect* 2003; 53: 187–192.
9. Rupnik M, Dupuy B, Fairweather NF *et al.* Revised nomenclature of *Clostridium difficile* toxins and associated genes. *J Med Microbiol* 2005; 54: 113–117.
10. Pituch H, Rupnik M, Obuch-Woszczatynski P, Grubesic A, Meisel-Mikolajczyk F, Luczak M. Detection of binary-toxin genes (cdtA and cdtB) among *Clostridium difficile* strains isolated from patients with *C. difficile*-associated diarrhoea (CDAD) in Poland. *J Med Microbiol* 2005; 54: 143–147.
11. Goncalves C, Decre D, Barbut F, Burghoffer B, Petit JC. Prevalence and characterization of a binary toxin (actin-specific ADP-ribosyltransferase) from *Clostridium difficile*. *J Clin Microbiol* 2004; 42: 1933–1939.

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12. Terhes G, Urban E, Soki J, Hamid KA, Nagy E. Community-acquired *Clostridium difficile* diarrhea caused by binary toxin, toxin A, and toxin B gene-positive isolates in Hungary. *J Clin Microbiol* 2004; 42: 4316–4318.
13. Loo VG, Poirier L, Miller MA *et al.* A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. *N Engl J Med* 2005; 353: 2442–2449.
14. Barbut F, Decré D, Lalande V *et al.* Clinical features of *Clostridium difficile*-associated diarrhoea due to binary toxin (actin-specific ADP-ribosyltransferase)-producing strains. *J Med Microbiol* 2005; 54: 181–185.
15. Alfa M, Kabani A, Lyerly D *et al.* Characterization of a toxin A-negative, toxin B-positive strain of *Clostridium difficile* responsible for a nosocomial outbreak of *Clostridium difficile*-associated diarrhea. *J Clin Microbiol* 2000; 38: 2706–2714.
16. Martin H, Willey B, Low DE *et al.* Characterization of *Clostridium difficile* strains isolated from patients in Ontario, Canada, from 2004 to 2006. *J Clin Microbiol* 2008; 46: 2999–3004.
17. Paltansing S, van den Berg RJ, Guseinova RA, Visser CE, van der Vorm ER, Kuijper EJ. Characteristics and incidence of *Clostridium difficile*-associated disease in The Netherlands, 2005. *Clin Microbiol Infect* 2007; 13: 1058–1064.
18. Warny M, Pepin J, Fang A *et al.* Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. *Lancet* 2005; 366: 1079–1084.
19. Goorhuis A, Van der Kooi T, Vaessen N *et al.* Spread and epidemiology of *Clostridium difficile* polymerase chain reaction ribotype 027/toxinotype III in The Netherlands. *Clin Infect Dis* 2007; 45: 695–703.
20. Kuijper E, van den Berg R, Debast S *et al. Clostridium difficile* ribotype 027, toxinotype III, the Netherlands. *Emerg Infect Dis* 2006; 12: 827–830.
21. Hubert B, Loo V, Bourgault A *et al.* A portrait of the geographic dissemination of the *Clostridium difficile* North American pulsed-field type 1 strain and the epidemiology of *C. difficile*-associated disease in Quebec. *Clin Infect Dis* 2007; 44: 238–244.
22. MacCannell D, Louie T, Gregson D *et al.* Molecular analysis of *Clostridium difficile* PCR ribotype 027 isolates from Eastern and Western Canada. *J Clin Microbiol* 2006; 44: 2147–2152.
23. Songer JG, Trinh HT, Killgore GE, Thompson AD, McDonald LC, Limbago BM. *Clostridium difficile* in retail meat products, USA, 2007. *Emerg Infect Dis* 2009; 15: 819–821.
24. Jhung MA, Thompson AD, Killgore GE *et al.* Toxinotype V *Clostridium difficile* in humans and food animals. *Emerg Infect Dis* 2008; 14: 1039–1045.
25. Pépin J, Valiquette L, Alary M *et al. Clostridium difficile*-associated diarrhea in a region of Quebec from 1991 to 2003: a changing pattern of disease severity. *CMAJ* 2004; 171: 466–472.
26. Kallen AJ, Thompson A, Ristaino P *et al.* Complete restriction of fluoroquinolone use to control an outbreak of *Clostridium difficile* infection at a community hospital. *Infect Control Hosp Epidemiol* 2009; 30: 264–272.
27. Centers for Disease Control and Prevention. Severe *Clostridium difficile*-associated disease in populations previously at low risk—four states, 2005. *MMWR* 2005; 54: 1201–1205.
28. Wilcox MH, Mooney L, Bendall R, Settle CD, Fawley WN. A case-control study of community-associated *Clostridium difficile* infection. *J Antimicrob Chemother* 2008; 62: 388–396.
29. Goorhuis A, Bakker D, Corver J *et al.* Emergence of *Clostridium difficile* infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. *Clin Infect Dis* 2008; 47: 1162–1170.
30. Rupnik M, Widmer A, Zimmermann O, Eckert C, Barbut F. *Clostridium difficile* toxinotype V, ribotype 078, in animals and humans. *J Clin Microbiol* 2008; 46: 2146.
31. Songer J, Jones R, Anderson M, Barbara A, Post K, Trinh H. Prevention of porcine *Clostridium difficile*-associated disease by competitive exclusion with nontoxigenic organisms. *Vet Microbiol* 2007; 124: 358–361.
32. Yaeger MJ, Kinyon JM, Glenn Songer J. A prospective, case control study evaluating the association between *Clostridium difficile* toxins in the colon of neonatal swine and gross and microscopic lesions. *J Vet Diagn Invest* 2007; 19: 52–59.
33. Hammitt M, Bueschel D, Keel M *et al.* A possible role for *Clostridium difficile* in the etiology of calf enteritis. *Vet Microbiol* 2007; 127: 343–352.
34. Rodriguez-Palacios A, Stämpfli H, Duffield T *et al. Clostridium difficile* PCR ribotypes in calves, Canada. *Emerg Infect Dis* 2006; 12: 1730–1736.
35. Rodriguez-Palacios A, Stämpfli H, Stalker M, Duffield T, Weese J. Natural and experimental infection of neonatal calves with *Clostridium difficile*. *Vet Microbiol* 2007; 124: 166–172.
36. Zidaric V, Zemljic M, Janezic S, Kocuvan A, Rupnik M. High diversity of *Clostridium difficile* genotypes isolated from a single poultry farm producing replacement laying hens. *Anaerobe* 2008; 14: 325–327.
37. Weese JS, Friendship R, Wakeford T, Zwambag A, Rousseau J, Reid-Smith R. 19th European Congress of Clinical Microbiology and Infectious Diseases, Abstract p1373.
38. Norman KN, Harvey RB, Scott HM, Hume ME, Andrews K, Brawley AD. Varied prevalence of *Clostridium difficile* in an integrated swine operation. *Anaerobe* 2009 [Epub ahead of print].
39. Alvarez-Perez S, Blanco JL, Bouza E *et al.* Prevalence of *Clostridium difficile* in diarrhoeic and non-diarrhoeic piglets. *Vet Microbiol* 2009; 137: 302–305.
40. Costa M, Arroyo L, Staempfli H, Weese JS. 19th European Congress of Clinical Microbiology and Infectious Diseases, 2009, Abstract p2041.
41. Debast SB, van Leengoed LA, Goorhuis A, Harmanus C, Kuijper EJ, Bergwerff AA. *Clostridium difficile* PCR ribotype 078 toxinotype V found in diarrhoeal pigs identical to isolates from affected humans. *Environ Microbiol* 2009; 11: 505–511.
42. Broda DM, DeLacy KM, Bell RG, Braggins TJ, Cook RL. Psychrotrophic *Clostridium* spp. associated with 'blown pack' spoilage of chilled vacuum-packed red meats and dog rolls in gas-impermeable plastic casings. *Int J Food Microbiol* 1996; 29: 335–352.
43. Weese J, Rousseau J, Arroyo L. Bacteriological evaluation of commercial canine and feline raw diets. *Can Vet J* 2005; 46: 513–516.
44. Rodriguez-Palacios A, Staempfli H, Duffield T, Weese J. *Clostridium difficile* in retail ground meat, Canada. *Emerg Infect Dis* 2007; 13: 485–487.
45. Rodriguez-Palacios A, Reid-Smith RJ, Staempfli HR *et al.* Possible seasonality of *Clostridium difficile* in retail meat, Canada. *Emerg Infect Dis* 2009; 15: 802–805.
46. Metcalf DS, Reid-Smith RJ, Avery BP, Weese JS. 19th European Congress of Clinical Microbiology and infectious Diseases, 2009, Abstract p2040.
47. Weese JS, Avery B, Rousseau J, Reid-Smith R. 19th European Congress of Clinical Microbiology and Infectious Diseases, 2009, Abstract p1374.
48. Weese JS, Avery B, Rousseau J, Reid-Smith R. 19th European Congress of Clinical Microbiology and Infectious Diseases, 2009, Abstract p2037.
49. Indra A, Lassnig H, Baliko N *et al. Clostridium difficile*: a new zoonotic agent? *Wien Klin Wochenschr* 2009; 121: 91–95.
50. Akerlund T, Persson I, Unemo M *et al.* Increased sporulation rate of epidemic *Clostridium difficile* type 027/NAP1. *J Clin Microbiol* 2008; 46: 1530–1533.

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51. Vengust M, Arroyo L, Weese J, Baird J. Preliminary evidence for dormant clostridial spores in equine skeletal muscle. *Equine Vet J* 2003; 35: 514–516.
52. al Saif N, Brazier JS. The distribution of *Clostridium difficile* in the environment of South Wales. *J Med Microbiol* 1996; 45: 133–137.
53. Bakri M. *Clostridium difficile* in ready to eat salads, Scotland. *Emerg Infect Dis* 2009; 15: 817–818.
54. Mutlu E, Wroe AJ, Sanchez-Hurtado K, Brazier JS, Poxton IR. Molecular characterization and antimicrobial susceptibility patterns of *Clostridium difficile* strains isolated from hospitals in south-east Scotland. *J Med Microbiol* 2007; 56: 921–929.
55. Weese JS, Finley R, Reid-Smith R, Janecko N, Rousseau J. 19th European Congress of Clinical Microbiology and Infectious Diseases, 2009, Abstract p2039.
56. Borriello S, Honour P, Turner T, Barclay F. Household pets as a potential reservoir for *Clostridium difficile* infection. *J Clin Pathol* 1983; 36: 84–87.
57. Clooten J, Kruth S, Arroyo L, Weese JS. Prevalence and risk factors for *Clostridium difficile* colonization in dogs and cats hospitalized in an intensive care unit. *Vet Microbiol* 2008; 129: 209–214.
58. Lefebvre S, Waltner-Toews D, Peregrine A *et al.* Prevalence of zoonotic agents in dogs visiting hospitalized people in Ontario: implications for infection control. *J Hosp Infect* 2006; 62: 458–466.
59. Hutin Y, Casin I, Lesprit P *et al.* Prevalence of and risk factors for *Clostridium difficile* colonization at admission to an infectious diseases ward. *Clin Infect Dis* 1997; 24: 920–924.
60. Muto CA. Asymptomatic *Clostridium difficile* colonization: is this the tip of another iceberg? *Clin Infect Dis* 2007; 45: 999–1000.
61. Pirs T, Ocepek M, Rupnik M. Isolation of *Clostridium difficile* from food animals in Slovenia. *J Med Microbiol* 2008; 57: 790–792.
62. Simango C, Mwakurudza S. *Clostridium difficile* in broiler chickens sold at market places in Zimbabwe and their antimicrobial susceptibility. *Int J Food Microbiol* 2008; 124: 268–270.
63. Keel K, Brazier J, Post K, Weese S, Songer J. Prevalence of PCR ribotypes among *Clostridium difficile* isolates from pigs, calves, and other species. *J Clin Microbiol* 2007; 45: 1963–1964.

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