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CLOSTRIDIUM DIFFICILE – A POTENTIALLY FOODBORNE ZOONOSE? SIGNIFICANCE IN HUMANS, ANIMALS AND FOOD

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Emergence of a hypervirulent variant of *Clostridium difficile* in Europe

Clostridium difficile is a gram positive, anaerobic, spore forming and toxin producing bacillus, which is widely distributed in the environment. It is present as part of the flora in the large intestine in only approx. 2% of healthy adults, but in 10-20% among the elderly population (Wilcox, 2003). The spores are resistant to heating, drying and chemical reagents including alcohol based disinfectants.

Epidemiology of *C. difficile* PCR ribotype 027

Since 2003, outbreaks of severe *Clostridium difficile*-associated diarrhoea (CDAD) with increased mortality rate have been caused by the emergence of a hypervirulent *C. difficile* strain in North America and Europe. Various typing methods are used to characterise this strain, which accordingly is referred to as toxinotype III and BI/NAP1/027 (restriction-endonuclease analysis group BI, pulsed-field gel electrophoresis North American PFGE [NAP] type1 and PCR ribotype 027) – in short: **CD027** (*C. difficile* PCR ribotype 027).

In Quebec, Canada the number of patients with severe CDAD has increased over the years: the incidence rose from 35.6/100.000 in 1991 to 156.3/100.000 in 2003 and for patients aged 65 years or more, it increased from 102.0 to 866.5 per 100.000 (Pepin, 2004). A high mortality was also noted among patients aged 65 years or more: 13.8% died within 30 days after diagnosis in 2003 compared to 4.7% in 1991 (Pepin, 2005a). Finally, a cohort study identified prior administration of fluoroquinolones and cephalosporins (especially second and third generation) to be the most important risk factor for CDAD in Quebec during the epidemic caused by the hypervirulent strain of *C. difficile* (Pepin, 2005b).

In 2008 CD027 was discovered in 16 European countries. Nine of them (UK, Netherlands, Belgium, France, Ireland, Luxembourg, Switzerland, Germany and Finland) reported outbreaks while seven (Austria, Denmark, Sweden, Norway, Hungary, Poland and Spain) reported sporadic cases. The true incidence of CD027 in Europe is difficult to estimate because national surveillance programmes are not fully implemented throughout the continent (Kuijper, 2008).

Clinical features

Clostridium difficile is recognized as the main aetiology of hospital-acquired infectious diarrhoea. However, because CDAD is underdiagnosed in the community setting the true incidence of community-acquired CDAD is unknown (Wilcox, 2003).
Clostridium difficile infections constitute 15-25% of all cases of antibiotic-associated diarrhoea (Bartlett, 2008). The manifestations of *C. difficile* acquisition range from asymptomatic carriage over mild diarrhoea to pseudomembranous colitis. The classical presentation of CDAD is non bloody, profuse diarrhoea accompanied by abdominal cramps and low-grade fever in patients who have been treated with antibacterial agents such as clindamycin, cephalosporins, broad spectrum penicillins and fluoroquinolones.
CD027 is characterised by an enhanced pathogenicity due to the production of Toxin A and Toxin B being 20 times higher than in common *C. difficile* strains (Warny, 2005). The unusual

severity of diarrhoea caused by CD027 enables more person-to-person transmission and a possibly enhanced ability to spread via fomites. CD027 is resistant to newer fluoroquinolones (e.g. moxifloxacin).

Virulence of CD027

Important virulence determinants seen for CD027 are the *C. difficile* toxins which are denoted Toxin A (enterotoxin), Toxin B (cytotoxin) and the binary toxin (CDT). The genes encoding Toxin A and Toxin B are located in a chromosomal pathogenicity locus whereas the binary toxin is encoded by the *cdtAB* operon. Furthermore, a 1 base pair deletion at position 117 in the toxin regulating gene (*tcdC*) is thought to increase the production of Toxin A and Toxin B. However, these virulence traits are also seen for other PCR ribotypes than 027 suggesting that national surveillance programmes should monitor not only CD027 but also other prevalent PCR ribotypes circulating in Europe.

Laboratory diagnosis

Common tests used for the diagnosis of *C. difficile* are: Immunoassays (IA) specific for Toxin A and Toxin B directly on stool specimens. Also faecal culture on selective medium (cycloserine cefoxitin fructose agar (CCFA)) followed by toxin detection either by IA or by PCR (Persson, 2008) on colonies (toxigenic culture) can be performed. The latter approach has the advantage of also being able to detect the binary toxin compared to IA which currently only includes detection of Toxin A and Toxin B.

Infection control in health care facilities

Once CDAD is diagnosed in a patient it is important to prevent further spread of *C. difficile*. This can be accomplished by patient isolation either in single rooms or by cohort isolation. Other measures which should be considered include: 1) environmental cleaning using sporicidal agents, e.g. hypochlorite solution, 2) elimination of selection pressure by discontinuing antimicrobial treatment if possible, and 3) hand hygiene: since bacterial spores are not killed by alcohols it is recommended to use soap based washing of the hands (Vonberg, 2008).

***Clostridium difficile* in animal husbandry, meat and other food**

In light of the last decades rise in human cases of *Clostridium difficile*-associated diarrhoea, it is increasingly important to investigate whether this pathogen is a foodborne zoonose. To date only a few studies have been carried out in this respect. It seems likely that an animal reservoir of *C. difficile* exists but yet there is no evidence that human *C. difficile* infections stem from such a reservoir.

***C. difficile* in household animals**

C. difficile-associated disease or asymptomatic carriage of *C. difficile* have been described from several animal species including horses, dogs, cats, monkeys, rabbits, hamsters, pigs and even ostriches and elephants (Arroyo,2005, Bojesen,2006). In horses a fecal prevalence of 2–29% have been reported, and in cats and dogs a prevalence of 6 to 40% emphasizing the widespread nature of *C. difficile* in animals

Erdemoglu et al. (2005) examined a group of cats and dogs brought to the veterinary clinic for the presence of *C. difficile* and Toxin A in feces. From 41% respectively 35% of dogs and cats suffering from enteritis, *C. difficile* were isolated. From another group of animals without enteritis, *C. difficile* was isolated from 29% of dogs and 29% of cats. Toxin A was detected in

28% of dogs with enteritis compared to 20% in dogs without enteritis. For cats, Toxin A was detected in 23 respectively 29% of the animals with and without enteritis.

Also in meat production animal as pigs, *C. difficile* can be frequently found. Songer (2004) reports numerous studies of isolating *C. difficile* in piglets in USA. One study reported 35% Toxin A-/Toxin B-positive *C. difficile* when examining 600 piglets with enteritis. In another study, fecal matter from 32 herds of piglets in North Carolina was examined and in 48% of these herds toxin positive *C. difficile* was isolated. Also Yager et al (2007) have reported high prevalence of *C. difficile* in pigs, as the study detected *C. difficile* in 50% of 129 piglets from USA. In many cases herds of piglets have suffered from clinical CDAD and mortality rates between 16% - 20% have been reported (Songer, 2004; Blasko & Bilkei, 2005).

Another often described syndrome in pig production is CDAD in post-parturient sows. After giving birth to a flock of piglets, sows often develop a syndrome called MMA (mastitis-metritis agalactiae) and are given antibiotic treatment. In some of the herds given antibiotic treatment, the sows subsequently developed CDAD and mortality rates between 13 - 16% were reported (Mauch & Bilkei, 2003, Kiss & Bilkei, 2004; Silvapru & Bilkei, 2005)

Rodriquez-Palacios et al (2006) describe the presence of *C. difficile* in calves from Canada. In this study, Rodriquez-Palacios examined feces from 144 calves having diarrhoea respectively 134 healthy calves for *C. difficile.* From 31 (11 diarrhoeal resp. 20 healthy) of the fecal samples, *C. difficile* was isolated, giving an overall prevalence of 11.2%. Of these 31 isolates 30 were subsequently shown to produce toxins.

C. difficile in meat and other food

Only a few studies regarding presence of *C. difficile* in meat/food have been carried out. Rodriquez-Palacios et al (2007) examined a total of 60 ground meat samples from retail outlets in Canada. From these 60 samples, 12 of 60 samples (20%) were found to contain *C. difficile* and 11 of the isolates were toxigenic. Eight of the 11 toxigenic *C. difficile* were toxinotype III.

In a more recent study, Rodriquez-Palacios et al (2009) examined 149 samples of ground beef and 65 samples of veal chops purchased at 210 Canadian retailers from January to August 2006. In contrast to their first study, these samples were analysed using 3 different methods and one of the methods was even performed in duplicate giving a total of 4 analysis pr. sample. The study found *C. difficile* in 10 of 149 (6.7%) of the ground beef samples and in 3 of 65 (4.6%) veal chop samples. The combined prevalence of *C. difficile* found in meat in this study was 6.1%, and isolates from 10 of 13 samples were toxigenic. However the agreement between the 4 analyses was very poor, as only two samples (ground beef) were found positive with more than one method. All other positive samples were only positive within one analysis. Consequently the diagnostic sensitivity of the methods was low, calculated to be between 23 and 39% compared to a diagnostic sensitivity for stool samples around 95%. The low diagnostic sensitivity can be explained by either low culture selectivity for the meat samples, or – more likely – by a very low number of *C. difficiles* spores present in the meat samples.

Glenn Songer et al (2009) examined meat (beef, pork and turkey) and ready-to-eat meat products from beef and pork collected from grocery stores in Tucson, Arizona from January to April 2007. A total of 37 samples of 88 (42%) were found to contain *C. difficile,* with no significant difference in prevalence between the meat species. The majority (25 isolates) be-

longed to PCR ribotype 078, but also PCR ribotype 027 toxinotype III was recovered from 4 samples.

Scott Weese et al (2009) examined another 230 samples of retail beef (115) and pork (115) from retailers in Canada. However, in this study the samples were analysed using both a qualitative method (enrichment) and a quantitative method (direct plating of 10-fold dilutions). The study found 12% of the ground pork as well as 12% of ground beef samples positive for *C. difficile*. The majority of the samples (20 of 28 positive) were positive only when using enrichment, indicating that the number of spores was low. The detection limit for the quantitative method (direct plating) was calculated to 10 spores/g. The remaining samples contained from 20 to 240 spores/g. Indeed, the low number of *C. difficiles* spores found in this study, support the hypothesis given by Rodriquez-Palacios et al (2009) as explanation for the poor diagnostic sensitivity obtained in their study.

Two other publications report of *C. difficile* in food from outside the North America. Jöbstl et al (pers. com) analysed 100 ground meat samples and 50 raw milk samples, collected in Austria, for *C. difficile*. In 3 samples of ground meat, *C. difficile* was isolated whereas none of the milk samples were found positive. Of the 3 *C. difficile* isolated, one was identified as a "human PCR ribotype", but no PCR ribotype 027 or 078 was found.
Bakri et al (2009) examined 40 ready-to-eat salads purchased in Glasgow supermarkets in 2008. Three (7.5%) of the salads (baby spinach, mixed leafy salad and lettuce) were positive for *C. difficile*, but only when using an enrichment based method indicating low level of contamination. One isolate was PCR ribotype 001, a common clinical isolate in Scotland, whereas the other two isolates were PCR ribotype 017.

C. difficile in the environment

Also studies of prevalence of *C. difficile* in environmental samples are seldom. Our literature survey disclosed only 2 publications, namely Al Saif & Brazier (1996) and Simango (2006) who examined environmental samples from Wales respectively Zimbabwe. *C. difficile* was frequently found in many different environmental samples as soil, well water, other water-samples (incl. swimming pools), veterinary clinics and less frequently found in family houses/residents and vegetables. A majority of these *C. difficile* isolates produced Toxin A.

Association between human and animal/meat isolates

Although studies have not been carried out systematically, the presence of toxigenic *C. difficile* and CDAD in household pets and meat-producing animal have been consistently reported. However, it is still questionable whether the *C. difficile* found in animal and meat is the actual source of human CDAD.

Some studies fail to demonstrate similarity between human and animal *C. difficile* types. Arroyo et al (2005) conducted a similarity study using isolates from horses, dogs and humans. Four of 20 human isolates belonged to the same PCR ribotype as 58 of 92 dog isolates and 5 of 21 horse isolates, suggesting a possible spread between these species. However, the majority of the human isolates (15 isolates) belonged to 5 different PCR ribotypes only found in human isolates.

Another study (Keel et al, 2007) compared North-American isolates from cattle, dogs, horses and pigs to human isolates. For pig and cattle isolates one PCR ribotype (078) predominated, comprising 94% of the cattle isolates and 83% of the pig isolates. Among the

human isolates included in this study, only one isolate belonged to PCR ribotype 078. In contrast, 6 PCR ribotypes were common for both human and horse isolates and 3 PCR ribotypes were common to human and dog isolates.

In contrast to these findings the two publications by Rodriquez-Palacios et al (2006, 2007) found strong correlations between the 12 isolates from retail ground meat respectively the 31 isolates from calves and the human isolates from Canada. For instance, 8 of the 12 isolates from ground meat were PCR ribotype M31 and toxinotype III with around 80% similarity to PCR ribotype O27. Of the remaining 4 isolates, 3 were identical to PCR ribotypes isolated from human cases.
In the cattle study, PCR ribotyping of the 31 isolates produced 8 different PCR ribotypes where 7 of these have been found in human cases. Nine of the 31 isolates were PCR ribotype O17, toxinotype VIII and 4 were PCR type O27, toxinotype III. Both PCR ribotypes have been reported in major human outbreaks in various countries.
Also the more recent publications by Scott Weese et al (2009) and Glen Songer et al (2009), found high prevalence of PCR ribotype 078 and 027 among the *C. difficile* isolated from meat samples. High prevalence of *C. difficile* PCR ribotype 078 was also found in a Dutch study (Debast et al, 2008) when examining diarrhoeal piglets but surprisingly not when examining healthy piglets. The high prevalence of *C. difficile* 078 in meat and pigs is of concern as this PCR ribotype is increasingly reported from human cases in Canada and the Netherlands.

Conclusion

It can be concluded, that pets and livestock animals frequently carry *C. difficile* in their gastro-intestinal system and thus may be a potential reservoir for clinical relevant strains eventually causing CDAD in humans. Increasing evidence of food, especially meat, being contaminated with clinical relevant *C. difficile*, although in low numbers, is nowadays presented in the literature. However, much more work is required to determine whether *C. difficile* contamination of retail meat is of clinical relevance, and results should be evaluated in context with studies finding *C. difficile* in treated water, vegetables, and in household environments. Exposure to low levels of *C. difficile* might be a common occurrence, with meat being just one of many possible sources. However, recent evidence of increasing rates of community acquired CDAD, continued identification of clinical relevant *C. difficile* strains in retail meat, mean that the potential risks should not be dismissed and that further study of meat and other food as a source of infection is warranted.
Also research in the ecology - survival, sporulation, germination and growth of *C. difficile* in food as meat, vegetables and milk are strongly requested.

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