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Is *Clostridium difficile*-associated infection a potentially zoonotic and foodborne disease?

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ABSTRACT

Clostridium difficile has received much attention in recent years because of the increased incidence and severity of nosocomial disease caused by this organism, but *C. difficile*-associated disease has also been reported in the community, and *C. difficile* is an emerging pathogen in animals. Early typing comparisons did not identify animals as an important source for human infection, but recent reports have shown a marked overlap between isolates from calves and humans, including two of the predominant outbreak types, 027 and 017. *C. difficile* has also been found in retail meat samples, suggesting that food could be involved in the transmission of *C. difficile* from animals to humans.

Keywords Animals, *Clostridium difficile*, disease, foodborne disease, meat, typing

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Clostridium difficile is a Gram-positive sporogenic anaerobic bacterium that can be a cause of intestinal disease, particularly following antibiotic treatment. It is usually considered to cause nosocomial infection, and most cases have been documented in the hospital environment [1]. Although the principal risk-factors are still prolonged hospitalisation, an age >65 years and antibiotic therapy, some recent changes in the epidemiology of *C. difficile*-associated disease have been observed. Overall, the incidence and severity of disease seem to be increasing [2], and reports of severe cases with a community onset are becoming more numerous. Furthermore, some of the community-acquired cases have occurred in a 'low-risk' population (i.e., young, without previous antibiotic therapy or previous hospitalisation) [3]. In addition, *C. difficile* has been recognised as an emerging animal pathogen [4].

There are several possible explanations for changes in the epidemiology of a pathogenic microorganism, including modification of the selection pressure in the existing environment (e.g., changes in the use of antibiotics in the hospital and non-hospital environments), the emergence of a novel variant or type of organism, and the introduction of a pathogen into/from a novel reservoir. The influence of antibiotic use on the incidence of *C. difficile* infections has been well-documented [5,6], and the way in which the epidemiology can change in response to a combination of antibiotic selection (fluoroquinolones) and the emergence of a new type is exemplified by recent outbreaks caused by type BI/NAP1/027 in the USA, Canada and some EU countries [1,6]. However, only part of the recent increase in mortality and morbidity caused by *C. difficile* infections can be accounted for by this new highly virulent type. Moreover, data published recently that compared human and animal isolates and revealed the presence of *C. difficile* in food now strongly suggest that animal reservoirs and transmission via foods are possible sources for community-associated infections.

Animals are an important source of human pathogenic microorganisms and can spread disease following direct or indirect contact, through environmental contamination or when used for food [7]. *C. difficile*-associated disease or asymptomatic carriage has been described in numerous animal species [4,8,9], but the *C. difficile* types in the human and the animal populations have not been compared in detail. The role of household pets as a potential reservoir for *C. difficile* infection

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was assessed as early as 1993, but no correlation was found between isolates from cats and dogs and isolates from humans in Australia [10], and it was concluded that these animals were not an important source of human infection at that time. As the human strains were isolated from the general population and not from the owners of the animals, no conclusion could be reached concerning possible household-related transmission between dogs/cats and humans.

Subsequent comparisons in Canada of small numbers of equine and dog isolates with human isolates have revealed that more than five ribotypes are found per host, and that different types are largely specific for each animal species. Only one of the ribotypes was detected in all three hosts; however, this ribotype accounted for 50% of all the isolates studied [11]. Colonisation of dogs with a highly virulent human strain and the potential role of dogs in transmission was exemplified by a hospital visitation dog that was colonised with epidemic type BI/NAP1/027 [12].

More recently, the same group of workers reported a surprisingly high degree of overlap in Canada between isolates from symptomatic and asymptomatic calves and recent human isolates [13]. All but one ribotype represented among isolates from calves had been recognised previously among isolates from humans. Indeed, two of the ribotypes identified have been associated with *C. difficile* outbreaks in humans, i.e., ribotype 017 (toxinotype VIII, $A^-B^+CDT^-$) and ribotype 027 (toxinotype III, $A^+B^+CDT^+$), while a third (078; toxinotype V) can be isolated readily from horses (personal unpublished results) and pigs (K. Keel, ClostPath 2003 Symposium, Woods Hole, MA, USA).

The changing prevalence of strains with the binary toxin gene could be an additional indication that animal and human reservoirs of *C. difficile* have started to overlap in recent years. Variant *C. difficile* strains (i.e., strains with changes in the PaLoc region encoding toxins TcdA and TcdB) usually encode a third toxin, binary toxin CDT [14]. In early studies, the prevalence of binary toxin-positive strains among human isolates was relatively low (generally between 1.6% and 10% in a non-outbreak situation) [15]. In contrast, the prevalence of binary toxin-positive strains among animal isolates appears to be much higher, ranging from 23% to 100%, with the lowest prevalence in horses (up to 43%), followed by piglets (up to 83%) and by cattle (up to 100%) (K. Keel, unpublished data; personal unpublished data). Thus, originally, it seemed that two rather separate populations of *C. difficile* existed and that binary toxin-positive strains were more likely to be associated with animals than with humans. However, in recent years, the proportion of binary toxin-positive strains in the human population has gradually increased. In addition to an interesting increase in susceptibility to some antibiotics, an Italian study of isolates from three different periods (before 1990, 1991–1999 and 2000–2001) revealed that the proportions of binary toxin-positive strains in the three periods were 0%, 24% and 45%, respectively [16]. Interestingly, the binary toxin-producing strains often seem to be associated with community-acquired *C. difficile* infections of such severity that hospitalisation is required [17,18]. Furthermore, binary toxin-positive isolates account for >50% of all isolates in some hospitals, sometimes because of an outbreak caused by type BI/NAP1/027, and sometimes because of the presence of a combination of several binary toxin-positive strains [19].

If animals are indeed a potential source of *C. difficile* infection, food could be one of the transmission routes from animals to humans. Approximately 20% of retail ground meat samples or other retail meat products have been shown to contain *C. difficile* [20] (G. Songer, ClostPath 2006 Symposium, Nottingham, UK), and at least some of the ribotypes found (077, 014, M26, M31) were the same as those already found among isolates from dogs, calves and humans. Type M26 is identical in a number of molecular characteristics (toxinotype III, 18-bp deletion in *tcdC*, presence of binary toxin) to type 027, and was also resistant to levofloxacin and clindamycin, but the ribotyping and pulsed-field gel electrophoresis profiles were only 80% similar to 027 strains from humans. *C. difficile* could contaminate meat during processing, but another possibility is that spores are already present in the muscle tissue. This latter possibility has been described for other clostridial species in horses, but not, to date, for *C. difficile* [21]. Nevertheless, an increasing number of studies suggest that *C. difficile* strains can be transferred between humans and animals. Whether animals could serve as an important

reservoir of *C. difficile*, with food as an important route of transmission, clearly needs further evaluation.

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