Clostridium difficile infection: the next big thing!

**Clostridium difficile** causes infectious diarrhoea in humans and animals. It has been found in pigs, horses, and cattle, suggesting a potential reservoir for human infection, and in 20–40% of meat products in Canada and the USA, suggesting the possibility of food-borne transmission. It is likely that excessive antimicrobial exposure is driving the establishment of *C. difficile* in animals, in a manner analogous to human infection, rather than the organism just being normal flora of the animal gastrointestinal tract. Outside Australia, PCR ribotype 078 is the most common ribotype of *C. difficile* found in pigs (83% in one study in the USA) and cattle (up to 100%) and this ribotype is now the third most common ribotype of *C. difficile* found in humans in Europe. Human and pig strains of *C. difficile* are genetically identical in Europe confirming that a zoonosis exists. Rates of community-acquired *C. difficile* infection (CDI) are increasing worldwide, and a new community strain of unidentified origin has recently emerged in Australia. Environmental contamination may also play a role. *C. difficile* spores survive in treated piggery effluent, the by-products of which are used to irrigate crops and pasture and manufacture compost. There is abundant evidence that food products intended for human consumption contain toxigenic strains of *C. difficile* but food-borne transmission remains unproven. Thus there are four problems that require resolution: a human health issue, an animal health issue and the factors common to both these problems, environmental contamination and antimicrobial misuse.

*Clostridium difficile* is an anaerobic, Gram-positive spore-forming bacterium and the leading cause of infectious diarrhoea in hospitalised humans, usually after disruption of colonic flora by antimicrobials. The major virulence factors are two toxins, TcdA (an enterotoxin) and TcdB (a cytotoxin), while the role of a third “binary” toxin is unknown. Contamination of the environment with *C. difficile* spores plays a critical role in transmissibility. Spores are resistant to many commonly used disinfectants and may remain in the environment for months.1 There has been a suggestion that *C. difficile* is a threat to Australia’s biosecurity.2 Now there is evidence that this warning has gone unheeded as rates of human *C. difficile* infection (CDI) increase dramatically3 and *C. difficile* is recovered from Australian production animals4,5.

**Human health**

The emergence of strains causing *C. difficile* infection in the community (CA-CDI) is becoming more commonplace.6 There is concern in Australia about the recent emergence of a novel highly virulent strain of *C. difficile* of community origin, the source of which has not been identified7-9. Unlike health care infections, CA-CDI is associated with younger, healthy people (particularly females), often without prior exposure to antimicrobials or contact with hospitalised patients10-13. Community strains differ from predominant hospital strains and are more heterogeneous, with many previously unidentified PCR ribotypes14. This suggests that other reservoirs of infection contribute to CA-CDI, possibly animals.

**Animal and food sources of *C. difficile***

*C. difficile* is a recognised enteric pathogen in a variety of animals including companion animals (cats, dogs, horses) and food animals (cattle, sheep, goats, pigs).15,16. In Australia *C. difficile* has been isolated from piglets, sheep, lambs, horses, cats, dogs, and cattle, with the highest prevalence in neonatal animals due to a lack of established gut flora at birth. For this reason predisposing antibiotics may not be required for development of CDI in young animals although there is anecdotal evidence in Australia of routine use of extended-spectrum cephalosporins in production animals. This is particularly concerning in the pork industry where gross contamination of facilities with *C. difficile* spores is commonplace. *C. difficile* can be isolated from the faeces of piglets one hour after birth, presumably ingested from their environment. Within 48 hours, 100% of piglets had acquired *C. difficile* of the same molecular type that was found in the piggery environment.17 A 2011 Australian study showed contamination with toxigenic *C. difficile* increased from 0% to 61% of sites

**Under the Microscope**

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within a swine facility only one month after occupation with pigs. Airborne *C. difficile* spores can be found up to 20 metres from the pig facility. The predominant genotype isolated from food production animals outside Australia is PCR ribotype 078, toxinotype V, NAP 7/8, REA group BK. This ribotype is now the third most common European human ribotype.

Overseas, meat products, seafood, ready-to-eat salads, salad leaves and vegetables are also contaminated with *C. difficile*, predominantly ribotype 078-like strains. Contamination may occur through spillage of gut contents at slaughter or direct contamination by food handlers during processing or retailing. Environmental contamination may also play a role. *C. difficile* spores are treated in piggy effluent, the by-products of which are then applied to agricultural land, used in retail compost manufacture, or recycled within the swine facility.

### Zoonotic transmission of *C. difficile*

Outside Australia, the increasing prevalence of PCR ribotype 078 in humans, food production animals and food products suggest potential zoonotic transmission. In the Netherlands, where infections with ribotype 078 increased more than fourfold from 2005 to 2008, patients infected with this ribotype were younger and acquired *C. difficile* in the community more frequently, particularly if they lived in rural pig producing areas. In the USA, the prevalence of ribotype 078 infections in humans has increased from 0.02% to 1.3% (pre-2001 to 2006) and ribotype 078 is increasingly associated with CA-CDI. These strains are indistinguishable or very closely related to animal ribotype 078 strains by PFGE analysis. Ribotype 078 strains from Dutch pigs and humans are indistinguishable by MVA subtyping. Transmission from humans to animals may also occur. *C. difficile* can be isolated from the faeces of hospital pet therapy dogs that had prior negative bacteriological cultures for *C. difficile*. These dogs were >2 times more likely to be colonised with *C. difficile* than dogs not visiting hospitals.

### Future

The Australian situation is less clear. Investigations are currently under way to identify the major strains of *C. difficile* causing community-onset infection and their origin. Interestingly, ribotype 078 has never been isolated from companion or production animals in Australia, and from very few human cases of CDI. Instead, we have found a variety of novel ribotypes and sequence types (STs) in animals, with some overlap of human strains. Many of these strains fall into clade 5, the clade of *C. difficile* that contains predominantly animal isolates. One, a pig strain, is genetically unique but MLST analysis shows it is similar to ribotype 078 (ST 11, clade 5). Much more work needs to be done; however, it is likely that the use of certain antimicrobials in production animals is resulting in amplification of *C. difficile* and subsequent spillover into humans by an as yet undetermined mechanism. While the organism will be new to many proponents of One Health, the concept will not be and it will require a multidisciplinary approach to prevent animal strains of *C. difficile* infiltrating human health systems.

References


Biographies

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