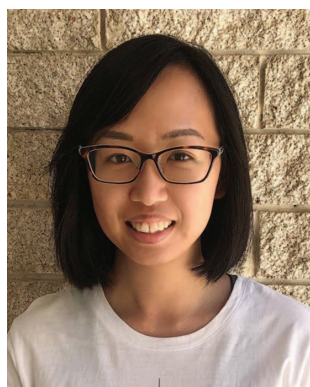


One Health: the global challenge of *Clostridium difficile* infection



Su-Chen Lim^A, Thomas V Riley^{A,B,C,D} and Daniel R Knight^{B,E}

^ASchool of Medical and Health Sciences, Edith Cowan University, Joondalup, WA, Australia

^BMedical, Molecular and Forensic Sciences, Murdoch University, Murdoch, WA, Australia

^CSchool of Biomedical Sciences, The University of Western Australia, Nedlands, WA, Australia

^DPathWest Laboratory Medicine, Department of Microbiology, Nedlands, WA, Australia

^ECorresponding author. Tel: +61 8 6457 4092, Email: daniel.knight@murdoch.edu.au

The One Health concept recognises that the health of humans is interconnected to the health of animals and the environment. It encourages multidisciplinary communication and collaboration with the aim of enhancing surveillance and research and developing integrative policy frameworks. *Clostridium difficile* (also known as *Clostridioides difficile*) infection (CDI) has long been viewed as a hospital-associated (HA) enteric disease mainly linked to the use of broad-spectrum antimicrobials that cause dysbiosis in the gut and loss of 'colonisation resistance'. However, since the early 2000s, the rate of community-associated CDI (CA-CDI) has increased to ~15% in Europe, ~30% in Australia and ~40% in the USA in populations often without obvious risk factors. Since the 1990s, it has become apparent that food animals are now a major reservoir and amplification host for *C. difficile*, including lineages of clinical importance. Cephalosporin antimicrobials, to which *C. difficile* is intrinsically resistant, were licensed for animal use in North America in 1990. By the second decade of the 21st century, there were reports of *C. difficile* contamination of food and the environment in general. Using whole-genome sequencing (WGS) and high-resolution typing, *C. difficile* isolates from humans, animals, food and the environment were proven to be genetically closely related

and, in some cases, indistinguishable. This suggests possible zoonoses and/or anthroponoses, with contaminated food and the environment acting as the conduit for transmission between animals and humans. This paper summarises the key evidence that demonstrates the One Health importance of *C. difficile*.

The role of asymptomatic carriers in the spread of *C. difficile*

In 2013, the landmark study of Eyre *et al.*¹ provided the first compelling evidence that asymptomatic carriers, and possibly other unknown sources external to the healthcare setting, were playing a major role in *C. difficile* transmission. The study, conducted in Oxfordshire in the United Kingdom used WGS and core-genome single nucleotide variant (cgSNV) analysis to examine *C. difficile* strains from 957 hospital- and community-identified CDI cases collected between 2008 and 2011¹. The authors found only 333 isolates (35%) had a clonal relationship (≤ 2 SNVs difference) with isolates of the same PCR ribotype (RT) from other CDI cases, and 428 isolates (45%) were genetically distinct with >10 SNVs difference in their core-genomes. Of the 333 cases with evidence of clonal transmission, only 126 (38%) had close hospital contact with another CDI patient and 120 (36%) had no plausible epidemiological link to a patient in the hospital system or community.

In early 2019, Sheth *et al.*² and Halstead *et al.*³ presented more evidence for asymptomatic carriers playing a part in the dissemination of *C. difficile* in hospitals. In both studies, asymptomatic patients were screened for *C. difficile* on admission and isolates were compared to isolates from symptomatic CDI patients using Multi-Locus Variable Number Tandem Repeat Analysis (MLVA) or cgSNV analysis. Of the 10–15% asymptomatic patients that tested positive for *C. difficile*, >80% were colonised by toxigenic *C. difficile* strains capable of causing disease^{2,3}. These studies revealed *C. difficile* transmission from asymptomatic patients to previously *C. difficile*-negative patients², and clustering of asymptomatic patients with symptomatic CDI patients³, supporting the idea that asymptomatic carriers are spreading *C. difficile*. Furthermore, in a separate study by Gonzalez-Orta *et al.*⁴, 27% of HA-CDI cases in Cleveland, USA, were infected with strains that the patients were previously colonised with on admission. This suggests that they were not true HA cases and that *C. difficile* was likely acquired in the community, with disease manifesting only after admission to hospital. With continuous importation of *C. difficile* into the hospital setting via asymptomatic carriers, community reservoirs are undoubtedly playing a much bigger role in the transmission of CDI than previously thought and the incidence of CA-CDI might have been grossly underestimated using the current guidelines⁵.

Community reservoirs

To date, *C. difficile* has been isolated from diverse array of sources/reservoirs including food animals (pigs, cattle, sheep and poultry), meat (veal, beef, pork, lamb, chicken and turkey), seafood (clams, salmon, shrimp and mussels), vegetables (lettuce, pea sprouts, ginger, carrots, potatoes and salad), the household environment (toilets, floors, bathroom sinks and soles of shoes) and the natural environment (rivers, lakes and soil)⁶. In summary, food animals, retail food and the environment are important reservoirs of *C. difficile*. The average prevalence of *C. difficile* in neonatal animals is always high, ranging from ~20% in calves to ~70% in piglets⁶. *C. difficile* prevalence as high as 42% in retail meat has been reported in the USA⁷; however, European studies reported a much lower figure of ~3%⁸, possibly related to differences in slaughtering practices. Meanwhile, the prevalence of *C. difficile* in natural environments such as soil and water averages ~30%⁶. The most common strains identified in these studies are *C. difficile* RT 014, belonging to multi-locus sequence types (MLSTs [STs]) 2, 13 and 49, and ST11 RTs 078, 126, 127 and 033. All these strains are toxigenic, associated with human CDI⁶, well established in multiple animal and environmental sources and invariably

resistant to numerous antimicrobials used in human and veterinary medicine^{9,10}. This further demonstrates the relevance of *C. difficile* to the One Health concept, i.e. there are three independent yet convergent problems that require an integrative solution: a human health issue, an animal health issue and an environmental issue.

Long-range interspecies transmission of *C. difficile*

To date, there has been no incontrovertible proof of foodborne or environmental transmission of *C. difficile*. Such proof remains elusive given *C. difficile* is not a typical foodborne or enteric pathogen: (1) not all individuals exposed to *C. difficile* will develop symptoms (depending on the vulnerability of their gut microbiota); (2) *C. difficile* is ubiquitous in the environment; and (3) the usual rules for source attribution are often not obeyed¹¹. Nevertheless, largely due to the advent of microbial genomics, there is now ample evidence that: (1) *C. difficile* common to humans and production animals share a recent evolutionary history; and (2) CDI has a substantial zoonotic component which results in the spillover of *C. difficile* into retail food and the environment. Building on their earlier work showing clonal transmission of *C. difficile* between a pig and a pig farmer¹², Knetsch *et al.*¹³ sequenced 247 *C. difficile* RT 078 strains from diverse sources in 22 countries across four continents (North America, Europe, Australia and Asia). Core-genome analysis revealed extensive clustering of human and animal strains, evidence of potential bidirectional spread of *C. difficile* between farm animals and humans. There was limited geographical clustering with clones of *C. difficile* RT 078 spread across towns, countries and continents. One clonal group of RT 078 showed intercontinental transmission between an animal in Canada and humans in the United Kingdom. Another study¹⁰, this time focusing on a global population of ST11, corroborated the findings of Knetsch *et al.*¹³ revealing a globally disseminated network of *C. difficile* ST11 clones (of RTs 078, 126, 127, 033 and 288) with the propensity for reciprocal zoonotic and/or anthroponotic transmission. Tetracycline use in agriculture and animal husbandry is widespread and its inappropriate use in the latter is well recognised. Dingle *et al.*¹⁴ found tetracycline selection to be a key driver of *C. difficile* RT 078 evolution, with multiple independent *tetM*-associated clonal expansions of this lineage occurring around the year 2000. Further supporting an agricultural focus for *C. difficile* RT 078, the evolutionary origins of these different tetracycline resistance elements were Tn916-like elements (which are capable of inter-species transfer) from established zoonotic species including *Streptococcus suis*, *Enterococcus faecalis* and *Escherichia coli*.

RT 014 is the most successful *C. difficile* lineage worldwide. In Australia, this RT 014 is well established in humans with CDI and pigs, accounting for around 30% and 25% of isolates, respectively^{15–17}. Knight *et al.*⁹ sequenced a contemporaneous collection of *C. difficile* RT 014 strains of human and porcine origin and cgSNV analysis revealed recent interspecies transmission, with 42% of human isolates having a clonal relationship with at least one animal isolate. Again, these clones were isolated months and thousands of kilometres apart across different States of Australia. Thus, it is unlikely that there was any direct contact between the animals and humans, however, it appears that *C. difficile* frequently moves between food animals and humans and that the zoonotic spread is not confined to any geographical region or local population. This strongly suggests an interconnected long-range zoonotic and/or anthroponotic transmission pathway involving recycled waste-products such as manure, biosolids and compost which could result in contaminated crops and/or widespread dissemination of *C. difficile* in the environment. Indeed, studies have shown that retail meat, vegetables, compost, public lawn, household environment and companion animals are reservoirs of clinically important and often antimicrobial-resistant (AMR) *C. difficile* lineages, including RT 014⁶. This is also in agreement with a WGS study involving 482 European hospitals which revealed no within-country clustering for RTs 078, 015, 002, 014 and 020, consistent with Europe-wide dissemination¹⁸.

Transmission cycle

How does *C. difficile* spread between food animals and humans with limited geographical clustering? The principal amplification hosts of *C. difficile* are animals, both human and non-human. *C. difficile* from food animals can contaminate meat during processing at the slaughterhouse and survive up to the point of human consumption as *C. difficile* spores can endure the recommended cooking temperature for meat (71°C) for over 2 h¹⁹ as well as freezing, chilling and disinfection processes^{20,21}. *C. difficile* spores can also disseminate in the air, in hospitals²² and animal production facilities²³. Transmission by invertebrate vectors also occurs. Depending on local agricultural practices and policies, manure from food animals can either be composted or applied directly onto farmland as fertiliser resulting in contamination of the farming environment. Even if the manure is composted, complete elimination of *C. difficile* spores is unlikely; ~60% of composted products such as garden mixes and mulches are contaminated with *C. difficile* (Lim *et al.* unpublished). Contaminated food waste can also be composted for use in gardening and landscaping. *C. difficile* can survive the process of sewage treatment²⁴ and release of treated

sewage effluent can impact rivers and marine life²⁵. While direct zoonotic transfer of *C. difficile* between pig farmers and pigs has been reported¹², for the general public indirect zoonotic transmission through food and the environment is more likely. With *C. difficile* being so widely disseminated in the community, household environments and companion animals are also being contaminated/colonised with *C. difficile*²⁶, providing yet another route for CDI transmission. Figure 1 shows the major reservoirs and known transmission pathways of CDI²⁷.

Conclusions

In summary, *C. difficile* is a pathogen with substantial community reservoirs and evidence of long-range interspecies transmission. This appears to be a recent (in the past 50 years) event, likely linked to anthropomorphic factors such as high-intensity animal husbandry, international travel and trade and, most critically, injudicious antimicrobial usage in farm animals. High-resolution One Health-focused surveillance of *C. difficile* from diverse human, animal and environmental sources will continue to be critical to the development of a better understanding of the epidemiological and genetic factors contributing to the emergence, evolution and spread of CDI. The control of CDI is currently focused on antimicrobial stewardship and infection control around CDI patients in the hospital setting. With the new knowledge of asymptomatic carriers spreading *C. difficile* in hospitals, early screening and isolating *C. difficile* carriers on hospital admission could help prevent HA-CDI as suggested in a recent Canadian study²⁸, which saw the incidence of HA-CDI decrease significantly from 6.9 to 3.0 per 10 000 patient-days, a 62.4% reduction in expected CDI cases. However, if we are to make meaningful interventions which impact upon both human and animal health, it is imperative that we move beyond the hospital setting and foster collaborative relationships between industry, government, veterinarians, clinicians and researchers. Enhanced antimicrobial stewardship in both human and veterinary settings is crucial but a more productive approach in reducing CDI would be to minimise the environmental burden of *C. difficile* by reducing carriage/infection in both animals and humans with a vaccine. Despite the recent demise of the Sanofi *C. difficile* vaccine program²⁹, several other contenders remain in the pipeline including Pfizer who are currently conducting a phase III trial of a vaccine based on genetically and chemically detoxified toxins A and B³⁰. In addition, a vaccine that offers protection against both CDI and colonisation (via mucosal antibodies to reduce the adhesion of *C. difficile* to mucus-producing intestinal cells) is currently being tested by GSK in a phase I clinical trial³¹. *C. difficile* is already considered a critical AMR pathogen by US Centers for Disease Control and Prevention³² and should also be

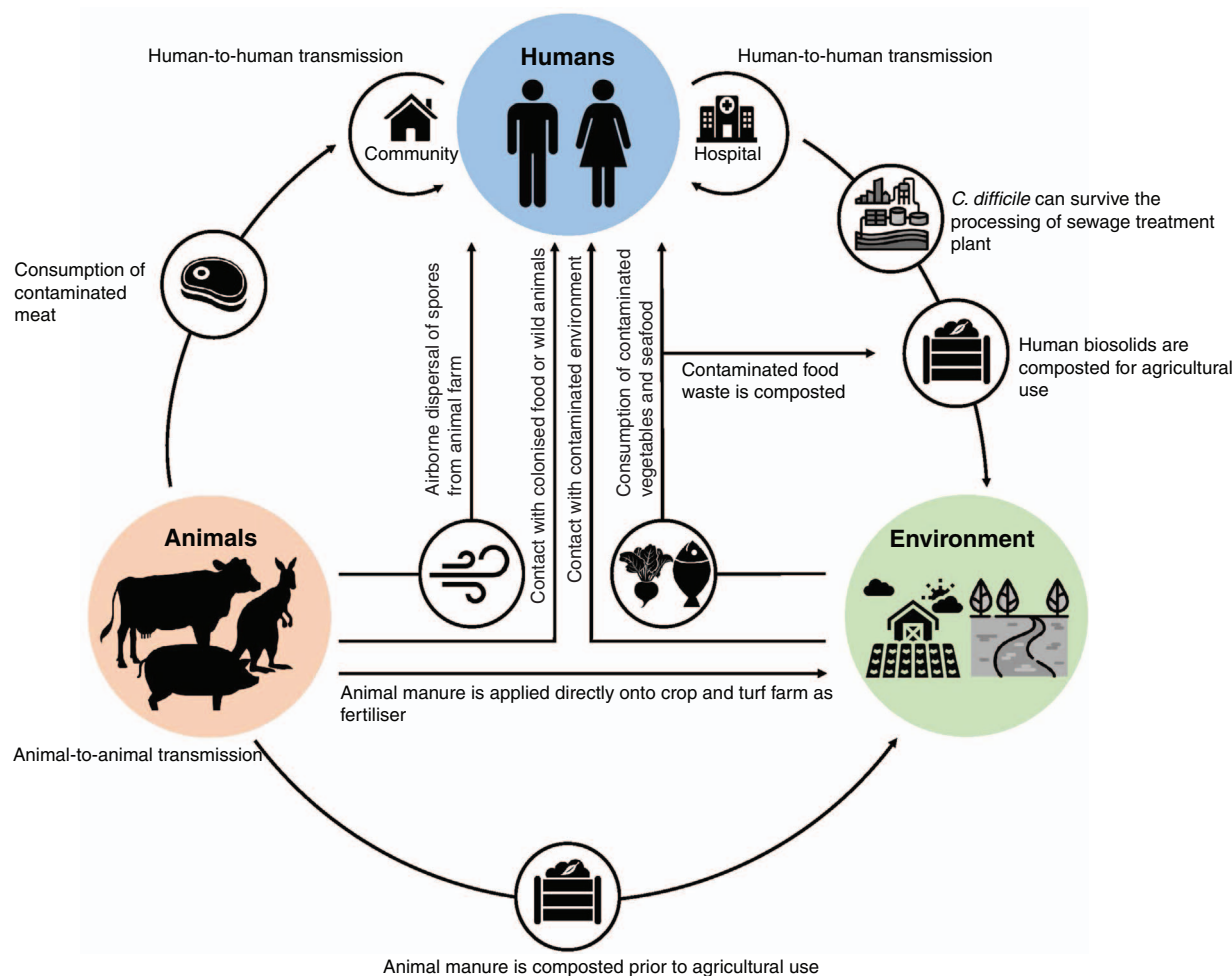


Figure 1. Transmission pathways for *C. difficile*.

recognised as the most significant One Health problem in the world today.

Conflicts of interest

The authors declare no conflicts of interest.

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Biographies

Su-Chen Lim is a postdoctoral research associate in the School of Medical and Health Sciences at Edith Cowan University. She completed her PhD at The University of Western Australia in 2019, investigating sources of *C. difficile* in the community of One Health importance. Her current work extends on this theme to determine the role of the contaminated environment in the transmission of *C. difficile*.

Tom Riley holds positions in various universities in Western Australia, as well as in Pathwest Laboratory Medicine. He has had a long-standing interest in healthcare-related infections, particularly the diagnosis, pathogenesis and epidemiology of CDI, in both humans and animals.

Daniel Knight is an NHMRC Early Career Research Fellow at Murdoch University in Western Australia. He has a background in AMR and infectious disease surveillance and currently manages a genomics-based research program focusing on evolutionary and One Health aspects of *C. difficile* Infection.

What's the difference ? Is it a worry?

An endemic disease is one that exists permanently in a particular region or population. Not usually seen by the media or the general public as a cause for concern even though it quietly and consistently claims lives.

An outbreak is the sudden occurrence of a disease in an unexpected location and/or in group in numbers greater than expected.

Seen as a possible news story and somewhat annoying but usually easily forgotten unless it evolves . . . into an epidemic.

An epidemic disease is one that suddenly involves a greater number of people than usual.

Seen as undesirable and unpleasant, prompting better hand hygiene by some. It too can evolve . . . into a pandemic.

A pandemic disease is one that causes epidemics worldwide.

Usually greeted with horror, mild hysteria and sometimes even panic.

The cause is suddenly a matter of grave concern, a killer, and the situation is very serious. Masks may be seen as mandatory street gear, worn for days without changing.