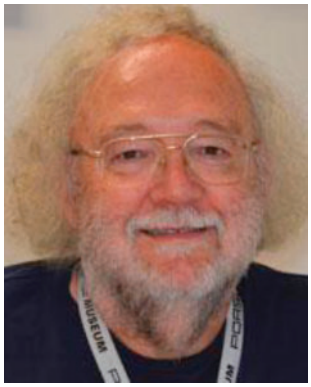
Community-acquired *Clostridium difficile* infection and Australian food animals





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***Clostridium difficile* is an anaerobic Gram positive spore-forming bacterium, the leading cause of infectious diarrhoea (*C. difficile* infection; CDI) in hospitalised humans. The assumption that CDI is primarily a hospital-acquired infection is being questioned. Community-acquired CDI (CA-CDI) is increasing[1] particularly in groups previously considered at low risk[2,3]. In Australia, CA-CDI rates doubled during 2011 and increased by 24% between 2011 and 2012[4]. Two potentially high-risk practices in Australian food animal husbandry may present a risk for CA-CDI: slaughtering of neonatal animals for food, and effluent recycling to agriculture.**

CA-CDI strains are genetically diverse, dominated by previously unidentified PCR ribotypes[5]. These strains often cause hospital outbreaks when patients are admitted with CDI from the community. A whole genome sequencing (WGS) study of isolates from 1250 patients with CDI at hospitals and in the community around Oxford, UK, found that 45% were genetically diverse and distinct from all previous human cases[6]. Recent local studies showed a range of unique PCR ribotypes (RTs) in humans not previously described in Australia or elsewhere[7,8]. Transmission of *C. difficile* has been linked to non-healthcare sources by molecular typing. In The Netherlands, WGS demonstrated RT 078 (toxinotype V, NAP 7/8, REA group BK) strains isolated from pigs and pig farmers were identical[9]. However, this is not surprising; RT 078 is the predominant genotype isolated from food production animals outside Australia[10], and this strain is now commonly isolated from human infections[11,12].

Increasing CA-CDI and genetic diversity of circulating *C. difficile* strains suggest a reservoir of *C. difficile* outside healthcare facilities. Similarity between community and animal strains has focussed attention on animals, or environmental sources common to animals and humans, as potential infection reservoirs.

C. difficile in animals and food

C. difficile is an enteric pathogen of companion animals (cats, dogs, horses) and food animals (cattle, sheep, goats, pigs)[13,14]. Neonates are typically colonised with *C. difficile* due to the lack of colonisation resistance afforded by mature intestinal microflora; hence prevalence decreases with age[15,16].

C. difficile spores contaminate retail meat and meat products outside Australia[10,17–23], ostensibly via gut contamination of carcases at slaughter. Food-borne transmission is possible as spores survive the recommended cooking temperature for ground meat (71°C)[24]. Salads and vegetables are also contaminated with *C. difficile* spores[14,25–27]. A plausible explanation for this is that *C. difficile* spores resist pond-based effluent treatment, the by-products of which are applied to agricultural land and used in compost manufacture; there is evidence for this in Australian livestock operations[28].

Potential sources of CDI in Australian food production animals

C. difficile is commonly found in Australian piglets, with 67% period prevalence in a study of neonatal herds[29]. These rates are higher than that reported in major pork-producing countries[30–32]. RT 078 has not been isolated from Australian piglets. Instead there is a heterogeneous mix of RTs, the majority of which (61%) have not been previously described in animals or humans. Piglet strains are overwhelmingly toxigenic (87%). Human and piglet RTs overlap but epidemiological links have not been determined.

Suckling piglets are not slaughtered for meat on a large scale, so the risk of carcass contamination is low. Contamination of the piggery environment with *C. difficile* spores poses a risk for spore dissemination however. Spore contamination in an affected farrowing unit is high (average: 4.08×10^5 spores/ pen in 82% of pens) (*M. Squire, in prep.*), likely a result of high-pressure hosing of sheds using treated liquid effluent. This is presumably true for other intensively farmed animal settings where *C. difficile* is endemic and effluent reuse occurs. Airborne spore dispersal and exposure of workers to bioaerosols could occur via pumping of raw effluent in open channels, use of treated liquid effluent for flushing under-pen gutters and irrigating crops/pasture, and tunnel ventilation of sheds. Manure storage facilities, compost bunds or treatment lagoons also provide the potential for bioaerosols to disseminate in high winds. Runoff from treatment ponds to local water courses and application of pond sludge to land are direct mechanisms of dispersal.

C. difficile prevalence in Australian cattle at slaughter ranges from 56% in veal calves <7 days of age to 1.8% in adult cattle[33]. This is higher than other cattle producing countries[34–38], possibly because of differences in slaughter age. Some Australian veal is slaughtered at <7 days compared with 21 weeks of age in North America, increasing the risk of carcass contamination with *C. difficile*. Recycled effluent from abattoirs processing veal calves and dairy feedlots also presents a risk. Three toxigenic RTs predominate (77%) in veal calves in Australia: RT127, RT033 and RT126. Along with RT 078, these genotypes form part of the genetically divergent clade 5[39]. These RTs have been isolated from humans with CDI in Australia although RT033 may be underreported as it is poorly detected by commonly used molecular tests[40].

Based on a small sample, sheep and lambs present a lower risk for CDI spillover with an overall prevalence rate of 4% (lambs 6.5% and sheep 0.6%)[41]; however, effluent treatment and reuse on intensive lamb finishing lots may present an opportunity for expansion and dissemination of *C. difficile*.

Conclusion

C. difficile is commonly isolated from food production animals in Australia, although prevalence is species- and age-dependent. Circumstantial evidence based on similarity of RT isolated from food animals, their effluent, and humans in the community suggests that spillover of *C. difficile* strains is occurring in Australia. Plausible avenues of transmission include effluent recycling and consumption of neonatal animals. Targeted research using highly-discriminatory WGS is required to confirm this. One stumbling block to learning more about CDI in animals is that most diagnostic tests used for laboratory diagnosis of CDI in humans do not perform well in animals[42]. Further work is required to address this problem.

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