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Review Article

Clostridium difficile Infection in Production Animals and Avian Species: A Review

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Abstract

Clostridium difficile is the leading cause of antibiotic-associated diarrhea and colitis in hospitalized humans. Recently, *C. difficile* infection (CDI) has been increasingly recognized as a cause of neonatal enteritis in food animals such as pigs, resulting in stunted growth, delays in weaning, and mortality, as well as colitis in large birds such as ostriches. *C. difficile* is a strictly anaerobic spore-forming bacterium, which produces two toxins A (TcdA) and B (TcdB) as its main virulence factors. The majority of strains isolated from animals produce an additional binary toxin (*C. difficile* transferase) that is associated with increased virulence. *C. difficile* is ubiquitous in the environment and has a wide host range. This review summarizes the epidemiology, clinical presentations, risk factors, and laboratory diagnosis of CDI in animals. Increased awareness by veterinarians and animal owners of the significance of clinical disease caused by *C. difficile* in livestock and avians is needed. Finally, this review provides an overview on methods for controlling environmental contamination and potential therapeutics available.

Introduction

Clostridium difficile is a strictly anaerobic Gram-positive bacillus that is the leading cause of antibiotic-associated diarrhea in humans, emerging as a significant cause of gastrointestinal infection in animals. An important property of *C. difficile* is its ability to form highly resistant spores that survive for a long time ($\sim$5 months) on contaminated surfaces (Kramer *et al.*, 2006). *C. difficile* infection (CDI) is transmitted by the fecal–oral route through the ingestion of these spores. The clinical presentation of CDI in humans and livestock varies from asymptomatic/subclinical carriage to mild diarrhea, severe diarrhea, and sometimes, life-threatening pseudomembranous colitis in humans (Hurley and Nguyen, 2002; Keessen *et al.*, 2011b). Disease arises due to the activity of two exotoxins TcdA and TcdB that are expressed in the gut by toxigenic strains of *C. difficile*. The presence of toxin receptors is required for toxin uptake by colonocytes (Keel and Songer, 2006). Different animal species vary in the type of toxin receptors present in the gut (Keel and Songer, 2006), but this does not correlate with disease severity. In addition, some *C. difficile* strains produce a binary toxin (*C. difficile* transferase, CDT) that has been associated with enhanced virulence in human disease (Schwan *et al.*, 2009). Some strains also differ in nucleic acid composition of *tcdC*, a toxin regulatory gene, which may result in an increased toxin production (Merrigan *et al.*, 2010). However, the significance of these latter features (CDT and *tcdC* mutations/deletions) continues to be debated (Carter *et al.*, 2011; Goldenberg and French, 2011).

The incidence and severity of CDI in humans have increased over recent years. In Europe and North America, this change has been attributed to the emergence since the early 2000s of a ''hypervirulent'' strain of *C. difficile*, ribotype (RT) 027 (NAP1/BI), which is fluoroquinolone resistant (Loo *et al.*, 2005). In addition, RT 078, a similarly virulent predominantly animal strain, is increasingly responsible for human infection in Europe (Goorhuis *et al.*, 2008; Bauer *et al.*, 2011). *C. difficile* RT 027 and RT 078 both produce all three toxins, TcdA, TcdB, and CDT (Merrigan *et al.*, 2010; Bauer *et al.*, 2011), and some strains have reduced susceptibility to various antibiotics used for treatment such as metronidazole (Álvarez-Pérez

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et al., 2013). Countries outside Europe and North America, including Australia, have seen a similar, but more recent rise in the incidence of CDI (Slimings *et al.*, 2014). Furthermore, CDI has been reported in people who have not been exposed to traditional CDI risk factors such as antibiotics, hospitalization, and living in an aged care facility (Khanna *et al.*, 2013).

Some strains of *C. difficile* recovered from different animal species and humans are indistinguishable by conventional molecular typing techniques, including polymerase chain reaction (PCR) ribotyping, multilocus sequence typing, and multilocus variable tandem repeat analysis (Goorhuis *et al.*, 2008; Bakker *et al.*, 2010; Marsh *et al.*, 2011; Janezic *et al.*, 2014; Knetsch *et al.,* 2014). More recently, this genetic overlap was further confirmed by whole-genome sequencing and core genome single-nucleotide polymorphism typing, which showed that pigs and pig farmers were colonized by indistinguishable strains of *C. difficile* (Knetsch *et al.*, 2014). These findings have raised concerns that cases of CDI could arise by zoonotic transmission. Transmission could occur by direct contact with live animals or their environment (Keessen *et al.*, 2013), and during or after slaughter, since *C. difficile* has been isolated from animals at slaughterhouses (Knight *et al.*, 2013) and also from retail meat (Houser *et al.*, 2012). The possibility that meat and meat products could play a role in human CDI although zoonotic transmission has not yet been conclusively proven. In addition, contamination of predominantly root vegetables has been reported to a lesser extent, despite early evidence of these foods as a potential source of *C. difficile* (Al Saif and Brazier, 1996).

Pathogenesis of and Host Susceptibility to CDI

C. difficile causes disease through expression of two main virulence factors, the toxins TcdA and TcdB (Merrigan *et al.*, 2010). The corresponding genes, *tcdA* and *tcdB*, are located on the chromosome alongside three accessory genes *tcdR*, *tcdC*, and *tcdE* that together form a 19.6-kb pathogenicity locus (PaLoc) (Britton and Young, 2014). Most toxigenic *C. difficile* isolates possess *tcdA* and *tcdB*, however, some variant isolates do not produce TcdA (Squire *et al.*, 2013) and others are missing the PaLoc altogether, instead having a 115 bp insertion.

TcdA has been described as an enterotoxin because it causes exudative colitis. TcdB is cytotoxic and causes epithelial cell collapse, apoptosis, and cell death. TcdA and TcdB are both large molecular weight toxins (308 and 209 kDa, respectively) belonging to the large clostridial toxin family (Jank and Aktories, 2008). Some strains of *C. difficile*, particularly those associated with livestock, produce binary toxin, the function of which remains speculative even though it has been associated with so-called ''hypervirulence'' (Kuehne *et al.*, 2014). It is thought to enhance microtubule protrusion from gut epithelial cells, leading to formation of a network of mesh around the bacterial cells resulting in adhesion (Schwan *et al.*, 2009).

As in humans, the intestinal microbiota is likely to play an important role in the susceptibility of animals to CDI. The intestinal microbiota prevents overgrowth of *C. difficile* and other enteric pathogens by competing for nutrition or acting as a mechanical blockade of enterocytes in a process that is often referred to as colonization resistance (Theriot and Young, 2015). Furthermore, the gut microbiome plays a role in the deconjugation of taurocholate to chenodeoxycholate, a key component in inhibiting spore germination in the small intestine and ceca (Giel *et al.*, 2010; Britton and Young, 2014), and the biosynthesis of secondary bile salts such as deoxycholate, which inhibit vegetative cell growth in the colon (Giel *et al.*, 2010; Theriot and Young, 2015). When the commensal intestinal microbiota has been disrupted, there is an increased production of cholate from bile salts that promotes spore germination (Giel *et al.*, 2010). This was recently demonstrated in a murine model and expanded to human studies. Mice treated with clindamycin developed an altered gut microbiome with a reduced ability to convert primary bile salts into secondary bile salts that correlated with the susceptibility to infection by *C. difficile* (Buffie *et al.*, 2015).

Disruption of the normal gut microbiota by antibiotics is the best known predisposing mechanism leading to *C. difficile* colonization of the large intestine (Theriot and Young, 2015). In particular, later generation cephalosporins, penicillins, carbapenems, clindamycin, trimethoprim/sulphonamides, and fluoroquinolones (in the United States) have been associated with greater risk (Slimings and Riley, 2014). Recently, an epidemiological study in veal calves found an association between antibiotic exposure and *C. difficile* shedding (Magistrali *et al.*, 2015), however, the association between antibiotic exposure and CDI has not been commonly reported in livestock as animals can develop diarrhea associated with antibiotic therapy that is unrelated to CDI.

Animals that rely on fermentation in the hindgut, known as pseudomonogastric animals (hamsters, horses, guinea pigs, and rabbits), are highly dependent upon commensal bacterial populations for digestion of fiber. Interestingly, most pseudomonogastrics are more susceptible to severe colitis and death associated with CDI compared with true monogastric animals (Keel and Songer, 2006). The evidence so far suggests that antibiotics may play a significant role in precipitating colonization of livestock by *C. difficile* and antibiotics may escalate CDI in animals without clinical disease. It is possible that the impact of antibiotics on the normal gut microbiota for pseudomonogastrics such as hamsters is more sudden than for true monogastric animals.

Clinical Signs of CDI

Intestinal colonization with *C. difficile*, and disease, is common in neonatal piglets within 7 days of farrowing (Norman *et al.*, 2009; Weese *et al.*, 2010b; Moono *et al.*, 2016). The most common clinical sign for CDI in livestock is diarrhea, which may be acute or chronic; however, many neonatal animals remain without clinical disease probably due to acquired colostral immunity (Squire and Riley, 2013). CDI may be self-limiting, intermittent, or continuous in nature. Piglets infected by *C. difficile* may present with a yellow pasty or watery, nonhemorrhagic diarrhea. Ostrich chicks often experience anorexia, weight loss (Shivaprasad, 2003), acute diarrhea, and sudden death within 3 days (Frazier *et al.*, 1993; Cooper *et al.*, 2013). CDI should be considered in the differential diagnosis in poultry, as one of many diarrhea-causing enteropathogens (Cooper *et al.*, 2013). Obstipation and constipation, scrotal edema, and dyspnea occur uncommonly in piglets (Steele *et al.*, 2010). In humans, CDI occurs in older people as neonates are thought not to have toxin

receptors. Clinical presentation in humans is similar to animals and patients may present with malaise, abdominal pain, nausea, anorexia, watery diarrhea, low-grade fever, and peripheral leukocytosis (Hurley and Nguyen, 2002).

Laboratory Diagnosis of CDI

The diagnostic tests available for detection of *C. difficile* in humans can broadly be classified into three categories (Crobach *et al.*, 2016). First, there are tests such as toxigenic culture to isolate toxin-producing *C. difficile*; second, tests that detect *C. difficile* products such as glutamate dehydrogenase (GDH) and toxins A and/or B; and last, tests that detects *C. difficile* genes.

Although toxigenic culture for *C. difficile* is labor intensive with a long turnaround time, it is still regarded as one of the gold standards for diagnosis of human CDI (Crobach *et al.*, 2016). Toxigenic culture involves isolating *C. difficile* from feces by using selective culture media and determining if the isolate is toxin producing (Burnham and Carroll, 2013; Lund and Peck, 2015). The methods for isolating *C. difficile* from feces either by direct plating on selective media and/or selective enrichment in broth, followed by plating on selective media, have been extensively reported (Lund and Peck, 2015). By direct culture, chromogenic agar (bioMérieux, Marcy l'Etoile, France) gives a shorter turnaround time (24 h) compared to prereduced cycloserine-cefoxitin-fructose agar with added sodium taurocholate (Carson *et al.*, 2013). Presumptive *C. difficile* colonies on blood agar are identified by chartreuse fluorescence under UV light (~360 nm wavelength), colonial morphology (ground glass appearance), and horse dung odor. Identification of uncertain isolates can be achieved by Gram staining and detection of L-proline aminopeptidase (Knight *et al.*, 2014), or more commonly, recently, MALDI-TOF-MS (Kim *et al.*, 2016).

Other assays for diagnosing CDI include commercially available enzyme immunoassays (EIA) (Crobach *et al.*, 2016). Despite the limitations associated with these tests (Tenover *et al.*, 2010; Burnham and Carroll, 2013), they are popular in laboratories because they are easy to use, relatively cheap, and have a short turnaround time. Some EIA are designed to detect GDH in feces, the ''common antigen'' on *C. difficile* strains, in addition to TcdA and TcdB, even though there are reports of reduced sensitivity for these tests (Tenover *et al.*, 2010). EIA that target GDH were initially said to have a higher sensitivity than those that only target TcdA or TcdB (Crobach *et al.*, 2016). Furthermore, some studies have suggested that EIA vary in their ability to detect certain RTs of *C. difficile* in human disease (Tenover *et al.*, 2010).

In addition, while there is no correlation between strain type, toxin in feces, and disease severity both in humans and animals (Yaeger *et al.*, 2002), a large study by Planche *et al.* (2013) showed that the presence of toxins in feces predicted poorer outcomes in humans. Although EIA that target GDH and PCR methods that detect toxin genes have relatively high sensitivity, they lack specificity for disease. Therefore, a two-step diagnostic algorithm has been suggested that involves retesting positive samples with a toxin EIA, which increases specificity and positive predictive value. A complete diagnosis of CDI in pigs or indeed other animal species will include a clinical history, toxigenic culture of *C. difficile*, and detection of free toxins in feces or detection of toxin genes or enzyme in isolates. Further, the European Society for Clinical Microbiology and Infectious Disease (ESCMID) recommends testing feces that are not formed and are negative for other enteropathogens (Crobach *et al.*, 2016). Currently, no single standalone diagnostic test for CDI with suitable sensitivity and specificity is available (Bloomfield and Riley, 2016).

Most of the CDI diagnostic tests available on the market have been validated for human medicine and these perform suboptimally on animal samples. For example, some human commercial molecular diagnostic assays showed low sensitivity in the range of 25% to 50% on animal samples (Knight *et al.*, 2014). The reason for suboptimal performance of molecular diagnostic tools in animal samples is unclear and requires further research. Better diagnostic tools are crucial for the early detection of many veterinary pathogens, including *C. difficile*.

Epidemiology of *C. difficile* in Production Animals

Although diarrhea is common in neonatal livestock, there are potentially many pathogens that may be involved apart from *C. difficile*, such as enterotoxigenic *Escherichia coli*, *C. perfringens*, *Coccidia* sp., *Cryptosporidium* sp., *Giardia* sp., and rotavirus, among others. In Australia (Squire *et al.*, 2013), Europe, and North America (Yaeger *et al.*, 2002; Hammitt *et al.*, 2008), it is rare for other pathogens to be present with *C. difficile* in fecal samples, suggesting that *C. difficile* alone was associated with diarrhea. However, the importance of screening for other pathogens when undertaking *C. difficile* surveys should not be ignored.

C. difficile in Pigs

The earliest published report of natural infection with *C. difficile* in swine was that of two piglets diagnosed with enterocolitis in the 1980s (Jones *et al.*, 1983). A decade later, there was a major outbreak of CDI at a farm in Canada with a weekly mortality rate in the range 7% to 58% in piglets aged 1–14 days (Waters *et al.*, 1998). *C. difficile* was isolated from feces and toxins were detected, however, strain types were not determined. Postmortem findings consistent with CDI, such as mesocolonic edema and typhlocolitis, were common. The significance of CDI in piglets became prominent after a 12-year surveillance study of enteric pathogens in neonatal pigs at the Iowa Veterinary Hospital. This study showed a decline in the relative frequency of traditional enteric pathogens such as transmissible gastroenteritis virus, *E. coli*, and *C. perfringens* type C from 70% to 21%, and an increase in *C. difficile* (55%) (Yaeger *et al.*, 2002).

The prevalence of *C. difficile* in piglets aged between 1 and 2 weeks has been reported in the range of 50% to nearly 100% in asymptomatic piglets (Keel and Songer, 2006; Weese *et al.*, 2010b; Moono *et al.*, 2016). This high prevalence is followed by a gradual decline as piglets grow older (Norman *et al.*, 2009; Weese *et al.*, 2010b; Moono *et al.*, 2016). Piglets infected with *C. difficile* (diarrheic) can be underweight by 10–15% and also have an extended weaning time (Songer and Uzal, 2005). Squire *et al.* (2013) reported a monthly mortality rate of 14% in piglets. Even though sporadic outbreaks of CDI in adult pigs are rare, they can have significant consequences because adult pigs can also die from infection (Kiss and Bilkei, 2005).

Risk factors for CDI in pigs

Few studies have adequately investigated the risk factors contributing to CDI in pigs. Pig age is the most commonly reported factor associated with risk of CDI. The prevalence of *C. difficile* is typically highest in piglets up to 7 days postpartum (Norman *et al.*, 2009; Weese *et al.*, 2010b; Moono *et al.*, 2016). Infection is likely to be acquired from the surrounding environment rather than by vertical transmission since piglets born by caesarean section were culture negative (Hopman *et al.*, 2011). A longitudinal study in the United States found higher *C. difficile* prevalence in cooler months (16.2%) than in warmer months (10.3%) in a vertically integrated pig farm (Norman *et al.*, 2009). Whether the seasonal variation in CDI seen in swine in North America is due to temperature, humidity, or other seasonal factors and whether this impacts exposure or host susceptibility are unclear. In addition, airborne dispersal of *C. difficile* spores in a piggery has been reported (Keessen *et al.*, 2011a).

Vermin may play a role in the spread of *C. difficile* on pig farms. A survey on a pig farm in The Netherlands was undertaken to determine whether mice (*Mus musculus linnaeus*) were competent vectors for *C. difficile* (Burt *et al.*, 2012). Mice on the farm were trapped and their skin, muscles, and gut contents aseptically sampled for *C. difficile*. In addition, dead insects (drain flies, lesser house flies, and yellow meal worms) and birds were also sampled. The external body surface of mice had a culture prevalence rate of 51–66% compared to 8% for the gastrointestinal contents, with the predominant strain of *C. difficile* being RT 078. Although Burt *et al.* (2012) did not sample pigs, the finding of RT 078 in vermin is significant because it is a well-established animal pathogen. Given that the contamination rate of the body surfaces was higher than the gut, mice may be more likely to spread *C. difficile* mechanically in the environment than through the fecal route. The prevalence of *C. difficile* in wild bird droppings was 4%, in dead sparrows 66%, and in various insects 56–100% (Burt *et al.*, 2012). *C. difficile* has since been isolated from urban rats, further highlighting the role vermin could play in dissemination of *C. difficile* in the environment (Himsworth *et al.*, 2014). Furthermore, a recent study showed that raccoons could play a role in *C. difficile* transmission at pig farms and the environment (Bondo *et al.*, 2015).

C. difficile in Cattle

C. difficile was first isolated from cattle in the early 1980s. Preweaning neonatal calf enteritis and mortality is a common problem in the cattle industry (Gunn, 2003). Although there are many pathogens associated with neonatal calf enteritis, *C. difficile* was only described as a potential causative agent in the early 2000s (Hammitt *et al.*, 2008). In addition, a study found a poor recovery rate of pathogens from feces of diarrheic calves; 25% to 45% samples did not yield any pathogen (Gunn, 2003). This study was limited by the small number of pathogens covered in the surveillance program and, in particular, there was no *C. difficile* detection protocol in place. Using culture, Rodriguez-Palacios *et al.* (2006) reported a *C. difficile* prevalence rate of 7.6% (11/144) in diarrheic calves and 15% (20/134) in control calves. Toxins were more likely to be detected in diarrheic calves, 39.6% (57/144) compared to 20.9% (28/134) in controls. In a subsequent study, Rodriguez-Palacios *et al.* (2007b) did not find an association between *C. difficile* colonization in calves and disease. Hammitt *et al.* (2008) reported a *C. difficile* prevalence of 25.3% (64/253) in feces of diarrheic calves compared to nondiarrheic calves, 13% (7/53). Furthermore, 22.9% (58/253) of specimens from diarrheic calves were toxin positive compared to 30.2% (16/53) from nondiarrheic calves (Hammitt *et al.*, 2008). Although idiopathic enteritis in calves aged 1–14 days is well described, few studies have screened for *C. difficile* and those that did failed to find a correlation between *C. difficile* colonization and disease.

In the dairy industry, male calves are considered surplus and used for veal production. They are either slaughtered quite young (<4 weeks) or kept for ~6 months. Longitudinal studies of veal calves showed that young animals were colonized soon after birth, with the prevalence gradually declining as the calves grew older (Costa *et al.*, 2011; Zidaric *et al.*, 2012; Houser *et al.*, 2012; Magistrali *et al.*, 2015). Zidaric *et al.* (2012) reported a great diversity of *C. difficile* strains in veal calves (RTs 078, 126, 012, 045, 010, and 033) similar to Costa *et al.* (2011), with this diversity diminishing as they grew older. A cross-sectional study of 7-day-old veal calves conducted in Australia found a *C. difficile* prevalence of 56% with three predominant RTs (126, 033, and 127) (Knight *et al.*, 2013). These RTs belong to clade 5 of *C. difficile*, as do RTs 078 and 237, and are frequently isolated from livestock and occasionally from human cases of CDI (Magistrali *et al.*, 2015; Tsai *et al.*, 2016). The high prevalence of *C. difficile* in calves could increase the risk of meat contamination at the abattoir.

Risk factors for CDI in cattle

Putative risk factors for CDI in cattle include younger age and antibiotic use. Magistrali *et al.* (2015) found that veal calves aged 13–28 days were twice as likely to shed *C. difficile* than those aged 36–45 days (odds ratio 4.57 vs. 2.79). Elsewhere, calves reached a peak of *C. difficile* shedding by at least 14–18 days of age (Costa *et al.*, 2011; Zidaric *et al.*, 2012). Antimicrobial use appears to be a common practice in veal production in Europe (Zidaric *et al.*, 2012; Magistrali *et al.*, 2015) and United States (Costa *et al.*, 2011). The use of multiple antimicrobials, or polymixin E, or a beta-lactam antimicrobial was highly associated with *C. difficile* shedding in veal calves (odds ratio 5.83) (Magistrali *et al.*, 2015). Interestingly, Costa *et al.* (2011) in the United States demonstrated no association between *C. difficile* shedding by calves and housing type, however, this requires further investigation as production systems for veal calves vary immensely within and between countries.

C. difficile in Goats and Sheep

Although there are few studies on the prevalence of *C. difficile* in sheep and goats (0–8.5%) (Knight and Riley, 2013; Avberšek *et al.*, 2015; Rodriguez *et al.*, 2016), the available literature does not suggest that they pose a major risk of CDI in humans.

C. difficile in Farmed Birds

Prevalence studies show that poultry can be colonized with *C. difficile* (Simango, 2006; Simango and Mwakurudza,

2008). In a cross-sectional study of poultry in Zimbabwe, the *C. difficile* culture prevalence was reported as 29% and, of the strains isolated, 90% were toxigenic (Simango and Mwakurudza, 2008). However, these prevalence studies did not state the age of the chickens sampled and neither was there evidence of enteritis in the poultry. In addition, *C. difficile* has been reported in captive ostriches (Frazier *et al.*, 1993; Shivaprasad, 2003).

Risk factors for CDI in farmed birds

CDI in avian species appears similar to the manifestation in other animal species (Frazier *et al.*, 1993; Shivaprasad, 2003). In one study, 19-day-old captive ostrich chicks treated with amikacin, piperacillin, and enrofloxacin were diagnosed with CDI (Shivaprasad, 2003). In another study, an outbreak of CDI in 9-day-old ostrich chicks treated with sulfamerazine in North America was reported (Frazier *et al.*, 1993). This is consistent with literature reporting the association of antibiotic therapy and CDI in humans (Slimings and Riley, 2014). Although *C. difficile* colonization has been reported in poultry (Simango, 2006; Simango and Mwakurudza, 2008), there are no reports of enteritis associated with *C. difficile* colonization. Cephalosporins, which have been associated with CDI amplification in humans, are widely used in poultry production in North America (Webster, 2009). The relationship between exposure to antibiotics in poultry and *C. difficile* shedding needs further investigation.

C. difficile in Food Animals and Foodborne CDI

The majority of research about *C. difficile* in food has been conducted on meat and meat by-products, particularly, beef, poultry, and pork (Songer *et al.*, 2009; Weese *et al.*, 2010a; Rahimi *et al.*, 2014; Varshney *et al.*, 2014). In these studies, the prevalence of strains of *C. difficile* associated with illness in hospitalized patients varied from high, predominantly in North America, to lower figures, usually in Europe. The common RTs from animal studies have been found in meat, suggesting that the contamination is occurring somewhere during processing, rather than from another external source (Rodriguez *et al.*, 2016). Contamination of meat likely results from gut content spillage during evisceration or perhaps accumulation of spores within abattoir environment (Houser *et al.*, 2012). However, the data on *C. difficile* are limited within the abattoir environment. A recent study found high counts of spores in feces of slaughtered 5- to 7-day-old veal calves with a median concentration of 2.5×10^4 cfu/mL (Knight *et al.*, 2016). Furthermore, 16.7% ($n = 25/150$) of the carcass samples were contaminated with the median count of spores detected 7 cfu/cm^2 (Knight *et al.*, 2016), suggesting that the abattoir environment could contribute to contamination of meat with *C. difficile* spores.

In North America (Varshney *et al.*, 2014) and elsewhere (Rahimi *et al.*, 2014), the most prevalent *C. difficile* strain detected in meat is RT 078, although earlier studies highlighted significant levels of RT 027 (Rodriguez-Palacios *et al.*, 2007a; Marsh *et al.*, 2011). In Australia, harvesting meat from neonatal animals has been identified as a potential risk for community-associated CDI (Squire and Riley, 2013). The prevalence of *C. difficile* in production animals declines as they age (Knight *et al.*, 2013) and meat from older animals may pose a minimal risk. This conclusion is supported by a recent study coordinated by the US Centers for Disease Control and Prevention showing no contamination of meat from adult animals (Limbago *et al.*, 2012). Nonetheless, future research should target the abattoir environment as it has great potential for risk reduction in the food chain with, for example, comprehensive disinfection protocols.

The use of effluent from animals on crops could contaminate vegetables (Squire and Riley, 2013). Al Saif and Brazier (1996) detected *C. difficile* in raw vegetables 20 years ago. In 2009, *C. difficile* was detected in ready to eat organic and nonorganic salads in Scotland (Bakri *et al.*, 2009). More recently, *C. difficile* has been detected in nonroot vegetables such as lettuce, green peppers and eggplant (Eckert *et al.*, 2013; Rodriguez-Palacios *et al.*, 2014). The overall prevalence of *C. difficile* in vegetables was reported as being up to 7.5% (Rodriguez-Palacios *et al.*, 2014). The reason for the variations in *C. difficile* prevalence in vegetables is unclear, although differences in culture methods may be an important contributor.

The infectious dose and host factors are critical for disease manifestation in susceptible hosts. The infectious dose of *C. difficile* for humans is unknown and CDI is complicated further by the requirement for an insult to the gut microflora to occur before exposure. In addition, the frequency and quantity of contaminated food ingested might be a higher risk than the prevalence of *C. difficile* in food, and even low levels of contamination may be sufficient to cause CDI. Last, to address potential confounders such as laboratory contamination with *C. difficile* (Marsh, 2013), highly discriminatory finger printing techniques like whole-genome sequencing should be used in future studies.

Control of *C. difficile* in the Veterinary Environment

C. difficile spores can persist in the environment for more than 5 months (Kramer *et al.*, 2006). Like other pathogenic organisms, lower temperatures (4–5°C), high humidity, and quantity of inoculum have been suggested as potential causes of persistence (Kramer *et al.*, 2006). Although still controversial, some studies have shown that epidemic strains of *C. difficile* have a higher sporulation capacity than nonepidemic strains and may persist in the environment longer (Merrigan *et al.*, 2010). However, a study conducted by Robinson *et al.* (2014) did not find a difference in the sporulation capacity between hypervirulent and nonhypervirulent strains. The thymidine synthase gene in RT 027 strains could confer a growth advantage for its competitive fitness (Robinson *et al.*, 2014), however, the majority of factors that enhance fitness among epidemic strains of *C. difficile* are unknown.

In the United States, Norman *et al.* (2009) reported *C. difficile* from human and swine composite sewage samples from closed integrated human and swine populations and a study in Australia found *C. difficile* in treated pig effluent (Squire *et al.*, 2011). There is a high likelihood that animal and human effluent can contribute to environmental contamination. Studies investigating the efficacy of disinfectants and treatment regimens for effluent in livestock operations to achieve better control of CDI are needed.

Many humans treated with antibiotics for CDI experience a recurrence of infection and this has accelerated the need to identify alternative treatment regimens, including therapy that uses fecal microbiota transplantation (Britton and

Young, 2014). In a recent study, Buffie *et al.* (2015) demonstrated that there is a specific microbiome that assists colonization resistance against infection by toxigenic strains of *C. difficile*. Harvey *et al.* (2006) showed that enterally fed neonatal piglets had better colonization resistance against *C. difficile* than those parenterally fed. Kim *et al.* (2014) showed that tigecycline altered microbiota balance of gnotobiotic piglets (increased *Proteobacteria* and reduced *Firmicutes*), but this did not predispose piglets to CDI. In contrast, others have found that mice treated with tigecycline or hamsters with clindamycin were susceptible to CDI, despite showing a similar shift in microbiota to piglets (Peterfreund *et al.*, 2012; Bassis *et al.*, 2014). A recent study using metagenomics showed that the underlying ecological dynamics of gut microbiome (i.e., intra and interspecies) communities are independent of host influence (Bashan *et al.*, 2016). Overall, it appears that no single community of microbiota determines the mechanism by which the gut provides colonization resistance (Theriot and Young, 2015), however, determining the beneficial components in the microbiota of production animals may be important.

There have also been studies of the potential benefits of probiotics (Schoster *et al.*, 2015; Arruda *et al.*, 2016), although they show varied performance against CDI in animals and humans (Collado *et al.*, 2005). In addition, vaccines for humans are at various stages of development, although none is currently available for livestock.

Conclusions

C. difficile is an important pathogen of humans and animals. The fact that indistinguishable strains of *C. difficile* have been detected from humans, animals, and crops irrigated with manure suggests that *C. difficile* could be acquired from a common source or zoonotically transmitted. The development of tools that can accurately diagnose CDI in livestock will be crucial in improving our understanding of the evolving epidemiology of CDI and thus its control. However, the most important issue is likely to be the misuse of antimicrobials in production animals that is driving the amplification of *C. difficile*. Some research should be directed toward understanding the functionality of various host microbiomes as a treatment option for many infectious diseases, including *C. difficile*. In addition, manure used on crops should be screened for *C. difficile* and appropriately treated to prevent community-acquired CDI. Surveillance of animal populations for *C. difficile* is needed to clarify the relationship between livestock-associated CDI, contamination of food or the environment, and human CDI. Sophisticated molecular techniques involving whole-genome sequencing will be required to prove these relationships. Ultimately, the promotion of a dialogue between physicians, veterinarians, and food scientists in the development of a One Health approach will be essential to control CDI.

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